VARIABILITY IN SUSCEPTIBILITY OF NESTLING PASSERINES TO PARASITES

by

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Abstract

Young birds face a diversity of nest-dwelling, blood-feeding ectoparasites and, in many altricial bird species, asynchronous hatching creates size hierarchies within broods. These hierarchies increase the morphological and physiological variance within a brood, and therefore, susceptibility to parasites may also vary predictably with the size hierarchy. Consequently, individuals can differ in their susceptibility to parasites. My thesis investigated how nestling susceptibility to parasites differs both among and within broods. First, I experimentally manipulated nestling susceptibility to parasites using an anti-parasite drug, ivermectin. Nestlings with reduced susceptibility benefitted both morphologically (faster growth of and longer flight feathers) and physiologically (higher haemoglobin concentrations), and also had marginally greater fledging success. Within broods, larger-sized nestlings were more likely to survive when they received the anti-parasite treatment. I performed a second experiment where I manipulated nestling haemoglobin concentration, a common physiological effect of blood-feeding parasites, to test whether reduced haemoglobin concentration could be the mechanism that causes changes in nestling morphology, behaviour, and physiology in response to increased parasitism. The haemoglobin treatment was combined with a parasite load-reduction treatment. The haemoglobin treatment temporarily prevented a normal increase in nestling haemoglobin concentration, while reduction of parasite load resulted in higher haemoglobin concentrations. Both treatments positively affected growth and length of flight feathers, while only the parasite reduction treatment increased fledging success, although only early in the breeding season. Lastly, I examined nestling susceptibility to parasites within the context of a particularly severe, two-day harsh weather event. Nestlings that received an anti-parasite treatment were more likely to survive the harsh weather event than non-experimental nestlings. Therefore, I demonstrated that

nestling susceptibility to parasites does vary among and within broods and plays an important role in determining nestling phenotype and survival.

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IAG and RDD designed the study. IAG performed the fieldwork. IAG analyzed the data with input from RDD. IAG wrote the manuscript with input from RDD.

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1. General Introduction

The interaction between parasites and their hosts can have important ecological and evolutionary consequences for both participants. By definition, parasites obtain nutrients from their host and therefore, hosts are expected to respond behaviourally (e.g., grooming; Clayton et al. 2010) or immunologically (e.g., Møller and Erritzøe 2002; Owen et al. 2009; King et al. 2011; Koop et al. 2013) to minimize the harm imposed by parasites. This 'resistance strategy', in which the host fights the parasite to reduce or eventually clear infection, can be costly. An alternative is the 'tolerance strategy', in which hosts bear the infection, but minimize damage caused by an overreacting immune response (i.e., immunopathology; Sorci 2013). Tolerance may be an effective strategy if a parasite causes little damage to a host, whereas resistance may be a more effective strategy if a parasite causes substantial damage (e.g., Budischak et al. 2017). Life-history theory predicts that an organism's fitness is dictated by trade-offs between traits, such as growth, reproduction, and survival (Roff 1992; Stearns 1992). Parasitism can alter these trade-offs and thus, hosts may also respond by modifying their reproductive effort (Forbes 1993; Saino et al. 2002). Likewise, parasites have adaptations that increase their reproductive success and chance of transmission, as well as reduce the negative effects of host defenses (e.g., Wikel 1996; Lawrie et al. 1999; McCoy et al. 2002; Møller et al. 2005). Therefore, a parasite's virulence (defined as the amount of damage caused by the parasite to the host (Frank 1996) or the fitness cost paid by an infected host (Read 1994)) will depend not only on the parasite, but also the host and the environment in which the interaction occurs (Alizon et al. 2009).

During the nestling stage, altricial birds are often exposed to a diversity of nest-dwelling, blood-feeding ectoparasites, such as mites, fleas, and larval blow flies. These parasites remove

energy and resources from nestlings and, as a consequence, parasitism is expected to have negative effects on nestlings. By experimentally manipulating parasite loads (methods reviewed in Hund et al. 2015), researchers have successfully identified cause-effect relationships between parasites and consequences for hosts in a way that observational studies cannot. For example, some studies have found no effect (or inconsistent effects) of parasitism on nestlings. No effect of parasitism may be observed when parasite loads are not high enough to significantly affect nestlings (e.g., McKilligan 1996), parents compensate for the energy nestlings lose to parasites (e.g., Christe et al. 1996a), or the severity of the effects of parasites depend on environmental conditions (e.g., Allander 1998). Furthermore, when parasites are transmitted vertically from parent to offspring, a parasite's fitness is more reliant on a host's reproductive success and therefore, will likely be less virulent compared to parasites transmitted horizontally between unrelated hosts (Clayton and Tompkins 1994). Some studies, however, have found that ectoparasites can negatively affect nestling morphological development (e.g., de Lope et al. 1993; López-Arrabè et al. 2014), and hence adult reproductive success (e.g., Møller 1991; Koop et al. 2011). Ectoparasites also can affect immunological (e.g., Saino et al. 1998; Szép and Møller 1999; de Coster et al. 2010; Martínez-de la Puente et al. 2013) and physiological traits of nestlings (McKilligan 1996; Carleton 2008; Harriman et al. 2014).

When morphological variation occurs among nestlings within broods, ectoparasites may benefit from preferentially feeding on particular nestlings within a brood. The 'tasty chick hypothesis' (Christe et al. 1998) suggests that because immunocompetence generally decreases with body condition, the smallest nestling will have the least defense against parasites and therefore, from the parasite's perspective, will be the easiest to exploit (the "tasty chick"). Preference by parasites for weak hosts with decreased immune response has been shown in some

cases (Roulin et al. 2003; Roberts et al. 2004). Furthermore, fleas had lower survival when feeding on larger nestling great tits (*Parus major*) in a brood as opposed to smaller nestlings (Gallizzi and Richner 2008). Interestingly, this effect was only observed when the mothers were exposed to fleas prior to egg-laying. Survival of fleas was also reduced when feeding on nestlings from broods with higher mean nestling mass (Gallizzi and Richner 2008). Preference for large nestlings, either because they provide greater nutritive value or have greater surface area for ectoparasites to occupy, has also been demonstrated (e.g., Darolová et al. 1997; Dawson and Bortolotti 1997; Walker and Rotherham 2011). Fecundity of fleas was increased after feeding on supplementally fed nestlings vs. nestlings that did not receive extra food (Tschirren et al. 2007). Alternatively, parasites may balance host nutritional value and immune response strength, preferring the middle nestlings of intermediate condition (Bedhomme et al. 2004; Lambrechts et al. 2006; O'Brien and Dawson 2009). Louse flies (*Crataerina melbae*) had higher survival when feeding on nestling Alpine swifts (*Apus melba*) of intermediate body condition, but had lower survival and took smaller blood meals when feeding on either food-deprived nestlings or nestlings supplemented with methionine to boost their cutaneous immune response (Bize et al. 2008).

1.1 Study species and study area

A migratory bird species, the tree swallow (*Tachycineta bicolor*), breeds throughout central and northern North America, although typically not nesting north of the tree line (Winkler et al. 2011), and overwinters in the southern United States, Mexico, and northern Central America (Winkler et al. 2011; Laughlin et al. 2013; Bradley et al. 2014). Agile flyers, tree swallows feed on aerial insects (Winkler et al. 2011), but will consume vegetable matter,

predominantly bayberries (wax myrtle, *Myrica* spp.), particularly during the non-breeding season (Beal 1918; Hausman 1927). The most common insects composing the diet of breeding adults and nestlings include Diptera (true flies), Odonata (dragonflies and damselflies), Ephemeroptera (mayflies), and Trichoptera (caddisflies; Winkler et al. 2011). As secondary cavity nesters, tree swallows nest in tree cavities excavated by other bird species, such as northern flickers (*Colaptes auratus*) and red-naped sapsuckers (*Sphyrapicus nuchalis*; Aitken and Martin 2007), but do display some flexibility in their nesting behaviour as demonstrated by their use of non-excavated nest sites, such as broken tree limbs, hollow stumps, and crevices behind bark (Aitken and Martin 2007, 2008), and reports of atypical nest sites, such as active ferries (Common 1942). Tree swallows also readily use human-made nest boxes. During the early breeding season, when competition over nest cavities is intense, territorial disputes, aerial attacks, and chases are frequent (Stutchbury and Robertson 1987) and physical fights can result in injury and death of adult birds (Leffelaar and Robertson 1985; Robertson et al. 1986; Lombardo 1986). Although males bring feathers to the nest for the lining, the female performs the majority of nest construction (Winkler et al. 2011). The mean clutch size in tree swallows ranges from 4.7 to 6.5 eggs, depending on the geographical location (Winkler et al. 2011). Only the female incubates the eggs and broods the altricial young, but both parents feed the nestlings. Nestlings typically fledge between 18 and 22 days post-hatch (Winkler et al. 2011). Tree swallows are highly tolerant of repeated disturbance from humans (Jones 2003), making them an excellent study species.

A holarctic group of species, avian blow flies (*Protocalliphora* and *Trypocalliphora* spp.) occur in their Nearctic range throughout Canada, Greenland, the United States, including Alaska, and parts of Mexico, favouring higher altitudes in the southern parts of their range (Sabrosky et

al. 1989). While adults are thought to feed on natural sugars (e.g., nectar), larvae are obligate parasites, feeding on the blood of altricial birds (Bennett and Whitworth 1991). The blow fly life cycle and its timing are closely associated with their bird hosts. Soon after the bird eggs hatch, female flies lay their eggs in the nest material or directly on nestlings (Sabrosky et al. 1989). Depending on the species of blow fly, the eggs take between 24 and 72 hours to hatch (Sabrosky et al. 1989; Bennett and Whitworth 1991). The resulting larvae proceed through three instars before pupating, spending a shorter period of time in the first two instars than the longer third instar. The entire length of time spent as larvae ranges from 7 to 15 days (Sabrosky et al. 1989). A non-feeding pre-pupal stage varies between 1 to 4 days prior to the pupal stage, which ranges from 9 to 36 days, depending on the species and temperature (Sabrosky et al. 1989). Adults, rather than pupae, are thought to enter diapause and overwinter (Sabrosky et al. 1989). Although species composition varies by geographic location, the most common species reported in tree swallow nests include *P. sialia, P. bennetti, T. braueri, P. occidentalis*, and *P. rugosa* (Roby et al. 1992; Dawson 2004; Dawson et al. 2005a; Gentes et al. 2007).

I studied two populations of tree swallows near Prince George, BC, Canada (53°N, 123°W). Located in north-central British Columbia, the area is characterized by mixed forests, composed primarily of trembling aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), lodgepole pine (*Pinus contorta*), and white (*Picea glauca*) and black spruce (*Picea mariana*). The two sites, colloquially referred to as Stewards and Western, had 139 and 60 nest boxes, respectively. Both sites were surrounded by a human-altered landscape, consisting of land used for agriculture (predominantly haying and livestock grazing) and forestry, interspersed with intact forest and bodies of water (wetlands and lakes).

1.2 General objectives

1.2.1 Nestling susceptibility to parasites: variation within and among broods

Nestlings can differ in their susceptibility to parasites both at the brood- and nestling-level. Variation in susceptibility to parasites among broods can be created by differences in parental care, such as nest site selection (e.g., Merino and Potti 1995), sanitation behaviour (e.g., Cantarero et al. 2013), and the quantity (e.g., Bouslama et al. 2002) and quality (e.g., Bańbura et al. 2004) of food provided. The sex ratio of a brood also can affect how susceptible a brood is to parasites if male-biased (e.g., Tschirren et al. 2003) or female-biased parasitism (e.g., O'Brien and Dawson 2013) occurs in a population. Like many traits, both genetics (e.g., Lambrechts et al. 2006) and the environment (Merino and Potti 1996) can also influence the susceptibility of broods to parasites.

Nestling susceptibility to parasites may also vary among siblings within the same nest, accentuated by within-brood size hierarchies. Occurring in many altricial bird species, these hierarchies become established due to incubation beginning prior to the final egg(s) in a clutch being laid (Clark and Wilson 1981; Mock 1984) and/or egg size varying over the laying sequence (Parsons 1970; Schifferli 1973; Rydén 1978; Slagsvold et al. 1984), and are then maintained throughout the nestling-stage by sibling competition (e.g., Leonard and Horn 1996) and food allocation by the parents (e.g., Cotton et al. 1999). Brood size hierarchies increase morphological variation within a brood, with later hatched nestlings being smaller and/or taking longer to reach adult size (Bancroft 1984; Lago et al. 2000). Because individuals in poor condition typically have low immunocompetence (Saino et al. 1997; Christe et al. 1998; Alonso-Alvarez and Tella 2001), immune function can also vary with hatching order and, as may be expected, studies have found that earlier hatched young mount a stronger immune response than

later hatched young (e.g., Müller et al. 2003). The opposite pattern, however, has also been observed in some species (e.g., barn swallows (*Hirundo rustica*); Saino et al. 2001), where late-hatched nestlings show a greater immune response than early-hatched nestlings. This may reflect higher levels of infection resulting in greater activation of the immune system. Alternatively, maternal effects may play a role with a female depositing more immunoglobulins and/or immune-stimulating micronutrients (e.g., carotenoids) into later-laid eggs (e.g., Buechler et al. 2002; Hargitai et al. 2006; Newbrey et al. 2008). These differences in morphological and immunological characteristics should affect nestling susceptibility to parasites, influencing the amount of resources a nestling has to invest in defense against parasites, as well as how attractive a nestling appears to a parasite. In tree swallows, an asynchronously hatching bird species that has size hierarchies within broods, it is not known whether certain nestlings along the size hierarchy are more susceptible to parasites.

In chapter 2, I present the results of a study conducted in 2016 and 2017 in which I experimentally reduced nestling susceptibility to parasites, using a broad spectrum, anti-parasite drug, ivermectin. I measured nestling morphology (growth rates and size near to fledging), physiology (haemoglobin concentration), and fledging success. This allowed me to test whether nestling susceptibility to parasites varied with hatching order in tree swallows, as well as examine how reduced parasite susceptibility affects nestling traits and fledging success.

1.2.2 Reduction of haemoglobin concentration by hematophagous ectoparasites and the consequences for nestlings

The intensity of infestation by haematophagous, nest-dwelling parasites has consistently been negatively correlated with nestling haemoglobin concentration across multiple bird species

(reviewed in Minias 2015), but less often with haematocrit (e.g., Szép and Møller 1999; Norcross and Bolen 2002; Carleton 2008). This pattern suggests that nestlings may be able to regenerate lost red blood cells quickly, but not haemoglobin (O'Brien et al. 2001). In fact, nestling red-rumped swallows *(Hirundo daurica)* exposed to parasites had *higher* haematocrit levels than parasite-free nestlings (Merino et al. 2001). Lower haemoglobin concentration reduces the oxygen-carrying capacity in birds (Minias 2015) and can affect fledgling survival during the more active post-fledging period (Clayton and Tompkins 1994; Streby et al. 2009). During the nestling stage, haemoglobin concentration has been positively correlated with size-corrected body mass (O'Dwyer et al. 2007; Lill et al. 2013) and fledging success (Kaliński et al. 2017), but also age (Bolton et al. 1999; Simmons and Lill 2006), brood size (Minias et al. 2015), and quality of diet (Pryke et al. 2011; Pryke and Rollins 2012). It is not known, however, whether haemoglobin concentration actually affects nestling traits, or if nestling traits simply co-vary with haemoglobin concentration due to a number of similar factors influencing both (e.g., quality of diet: Pryke et al. 2011; Pryke and Rollins 2012; Twining et al. 2016). Until this causative relationship between haemoglobin concentration and nestling phenotype is confirmed, it cannot be established whether or not the reduction in nestling haemoglobin concentration by haematophagous ectoparasites contributes to some of the other negative effects commonly associated with parasitism.

In chapter 3, I present the results of a study conducted in 2017 in which I experimentally reduced nestling haemmoglobin concentration, using phenylhydrazine hydrochloride (PHZ), in combination with a nest-heating treatment that killed all nest-dwelling ectoparasites. I measured nestling morphology (growth rates and size near to fledging), physiology (haemoglobin and β-hydroxybutyrate concentration), begging behaviour, and fledging success. This allowed me to

test the effects of reduced haemoglobin concentration on nestling traits, while controlling the ectoparasite environment.

1.2.3 Susceptibility to parasites within the context of variable weather conditions

Weather conditions can act in complex ways on the interaction between nestlings and ectoparasites. Although the negative effects of parasites on nestlings can be amplified in years with poor environmental conditions, such as lower temperatures and higher rainfall (Dufva and Allander 1996; Merino and Potti 1996; Allander 1998), the interplay between the environment and parasites and how they affect nestlings also can depend on the particular parasite species. For example, fleas had a greater negative effect on nestling pied flycatchers during a cold and rainy year, whereas larval blow flies had a greater impact during a warmer year (Merino and Potti 1996). This result is partly explained by the fact that poor weather can have detrimental effects on the nest-dwelling ectoparasites themselves. In the above study, mites, fleas and blow flies were all less prevalent in the cold and rainy year, but fleas did relatively well compared to mites and blow flies (Merino and Potti 1996).

For aerial insectivores, weather conditions have important consequences on their food source, with the availability of flying insects declining with temperature (Winkler et al. 2013; Arbeiter et al. 2016). In tree swallows, harsh periods of inclement weather negatively affect chick morphology (Dawson 2008) and increase mortality (Gentes et al. 2006; Winkler et al. 2013; Beck et al. 2015). During such periods, when food availability is low and nestlings are likely under-fed, nestlings may be more susceptible to parasites, because they have limited resources to mount an immune response (e.g., Saino et al. 1997) or respond behaviourally to deter parasites (e.g., moving when parasites attempt to attach; Hori et al. 1990). In support, a

greater number of larval blow flies occurred in eastern bluebird (*Sialia sialis*) nests when nestlings were experimentally food-deprived for 1.5–2.5 hours every other day compared to control nests (Pinkowski 1977). As periods of low food coincides with cooler temperatures in aerial insectivores, ectoparasites, which are poikilotherms, will also be less active during these periods and may feed on nestlings less. In Ithaca, NY, USA, Winkler et al. (2013) identified the critical temperature defining 'cold snap' effects on mortality of nestling tree swallows as 18.5°C. When temperatures declined below this critical point, decreases in flying insect availability and increases in nestling mortality were observed (Winkler et al. 2013). The relative importance of parasitism on nestling mortality during cold snaps compared to other factors that can reduce nestling survival (e.g., parental quality; Silva et al. 2008), however, has not been investigated.

In chapter 4, I present the results of a study conducted in 2016 in which a particularly harsh two-day weather event resulted in the death of over half of all broods. This provided a unique opportunity to explore factors that were most important in influencing survival of tree swallows during poor weather conditions. I measured characteristics of adults (morphology, plumage colour and initial reproductive investment), broods (size, age, relative asynchrony), nestlings (relative mass within the brood), and nest environment (anti-parasite treatments, using ivermectin and nest heating). This allowed me to test the effects of the anti-parasite treatments relative to the above factors to identify which were most influential to nestling and brood survival during harsh weather.

1.3 Significance

Aerial insectivores are declining across North America (Nebel et al. 2010) and are the fastest declining group of birds in Canada (NABCI-Canada 2012). Therefore, it is essential to

understand as much of their biology as possible. Specifically, my thesis research expands our knowledge of parasite-host relationships by not only demonstrating the importance of nestling susceptibility to parasites both within and among broods, but also exploring the consequences of a common effect of haematophagous ectoparasites, the reduction of haemoglobin concentration. I also examined the effects of parasites within the context of a harsh weather event, which is particularly relevant in the context of climate change and the predicted increase in frequency of extreme weather events (IPCC 2014). From an applied perspective, my effective use of ivermectin in nestling birds could contribute to the conservation toolkit.

2. Morphological and physiological benefits of an anti-parasite treatment are influenced by within-brood size variation in tree swallows (*Tachycineta bicolor*)

2.1 Abstract

In all organisms, susceptibility to parasites can differ among individuals. Young, nest-bound birds are exposed to a diversity of nest-dwelling ectoparasites that typically feed on their blood. Within broods, hatching asynchrony creates size hierarchies that result in morphological and physiological variation among nest mates, and susceptibility to parasites also may vary predictably with this size hierarchy. My objective was to use a broad spectrum, anti-parasite drug, ivermectin (IVM), to treat individual nestling tree swallows (*Tachycineta bicolor*) and assess how nestling susceptibility to parasites varied both within and among broods. Broods were either assigned to an IVM group, where half of the nestlings in a brood received IVM injections and half received control injections of pure sesame oil, or to a control group where all nestlings received oil injections. I found that the IVM treatment reduced parasite loads for broods as a whole, thereby benefiting all nestlings in IVM broods. Specifically, nestlings from IVM broods were heavier and had longer flight feathers near to fledging, grew ninth primary feathers faster, and had higher haemoglobin concentrations and increased fledging success. Variation in size within broods, however, influenced some results; mass near to fledging increased with relative brood asynchrony, but only under higher parasite loads (i.e., control broods), and the chance of an individual fledging increased with its relative within-brood size, but only under lower parasite loads (i.e., IVM broods). By experimentally manipulating nestling susceptibility to parasites, I have demonstrated variation in nestling response to an anti-parasite treatment both within and

among broods, and future studies should investigate the underlying mechanism for why certain nestlings along the brood size hierarchy are more susceptible to parasites.

2.2 Introduction

In all organisms, individuals can vary in their susceptibility to parasites. Young, nest-bound birds often are exposed to a diversity of nest-dwelling ectoparasites that feed on nestling blood (Rendell and Verbeek 1996). Various factors can influence the parasite susceptibility of nestlings, both within and among broods, such as parental care, environmental conditions, asynchronous hatching, and host selection by parasites.

To reduce the parasite load that their young will face, parents may actively select nest sites that have fewer parasites. If parasites do not have a strong negative effect on reproductive success, infestation levels may have no influence on nest selection (Orell et al. 1993), whereas if parasites have a strong effect on offspring, parents may preferentially select nests with low parasite loads (Merino and Potti 1995). Parents also may perform nest sanitation behaviours to reduce the parasite exposure of their young, increasing rates of sanitation when higher parasite loads occur (Tripet et al. 2002, Cantarero et al. 2013). In some species, parents adjust feeding rates and increase the amount of food brought to parasitized nestlings to increase their chance of survival (Wesołowski 2001, Bouslama et al. 2002), although some species favour self-maintenance and decrease or do not change feeding rate in response to higher parasite loads (Rogers et al. 1991, Møller et al. 1994). Alternatively, adults may alter diet composition rather than feeding rate, bringing their young higher quality food when parasitized (Bańbura et al. 2004).

Environmental factors also influence the severity of the effect of parasites on hosts. In years with poor weather conditions (e.g., cool temperatures and high rainfall), negative effects of parasites on nestlings can be amplified (Dufva and Allander 1996, Merino and Potti 1996). Environmental conditions, however, also shape populations of nest-dwelling parasites. For example, prevalence of larval blow flies, fleas and mites was reduced in the nests of pied flycatchers (*Ficedula hypoleuca*) during a cold and rainy year compared to two warmer years (Merino and Potti 1996)

Commonly observed in many altricial bird species, asynchronous hatching creates size hierarchies within broods (Clark and Wilson 1981, Mock 1984). Later hatched young are at a time disadvantage compared to earlier hatched siblings, with young that hatch first beginning to receive food and grow before their siblings exit the egg. Thus, later hatched nestlings typically are smaller and/or take longer to reach adult size (Bancroft 1984, Lago et al. 2000). In tree swallows (*Tachycineta bicolor*), nestlings that hatched later weighed less at their asymptote of growth and had shorter primary flight feathers at fledging than their nest mates that hatched earlier (Zach 1982). Size hierarchies not only increase morphological differences among siblings, but also immunological differences, which can then influence susceptibility of nestlings to parasites. For example, immune function depended on risk of parasitism, but not body condition, in earlier-hatching, senior nestling hoopoes (*Upupa epops*), whereas body condition was more important for predicting immune function of later-hatching, junior nestlings within broods (Martín-Vivaldi et al. 2006). This suggests that immune function was limited by resource availability in junior, but not senior, nestlings and demonstrates how asynchronous hatching and size hierarchies can play an important role in parasite susceptibility. Size hierarchies can either be maintained, exaggerated, or diminished by several processes, including egg quality (e.g., yolk

nutrients that positively affect immune function, such as carotenoids) varying with laying order in a predictable manner (e.g., Blount et al. 2002), parents preferentially feeding certain nestlings within a brood (e.g., Leonard and Horn 1996), and sibling competition (e.g., Parker et al. 1989).

In addition to susceptibility of hosts to parasites, another key factor in host-parasite interactions is whether a parasite selects among different hosts. For example, parasites may preferentially feed on nestlings with certain phenotypes. The 'tasty chick hypothesis' (Christe et al. 1998) suggests parasites will favour smaller nestlings in a brood, which will have the least defense against parasites due to poor body condition and immunocompetence, and so from the perspective of the parasite be "tasty" (e.g., Roberts et al. 2004, Gallizzi and Richner 2008). Alternatively, parasites may preferentially feed on the larger nestlings in a brood, either because they provide the greatest nutritive value or the largest surface area for ectoparasites to occupy (e.g., Dawson and Bortolotti 1997, Valera et al. 2004). Finally, parasites may balance the strength of the host's immune response with its nutritional value, selecting hosts of intermediate condition, preferring middle nestlings within a brood (e.g., O'Brien and Dawson 2009).

As a selective pressure, parasites can strongly affect hosts, both ecologically and evolutionarily, and host susceptibility to parasites can influence how these effects shape a species (e.g., Møller 1990; Spencer et al. 2005). My objective was to investigate how nestling susceptibility to parasites affected morphological and physiological traits, and fledging success of nestlings, both among and within broods of tree swallows. To experimentally investigate relationships between nestlings and nest-dwelling ectoparasites, most studies have manipulated parasite load by treating the nest (techniques reviewed in Hund et al. 2015), which alters the parasite environment for the brood as a whole. In contrast, I manipulated the susceptibility to parasites of individual nestlings using ivermectin, a broad-spectrum, anti-parasite drug, which

allowed me to examine individual variation in response to parasites more effectively. If a nestling's susceptibility to parasites influences how much resources it has to allocate to development, then nestlings that are less susceptible to parasites (i.e., ivermectin-injected nestlings) will be positively affected by the treatment compared to nestlings that are more susceptible to parasites (i.e., sham-injected nestlings). Specifically, I predicted that nestlings treated with ivermectin would be morphologically larger, have faster rates of growth, higher levels of haemoglobin, and be more likely to fledge successfully. If the ingestion of ivermectin via nestling blood causes mortality of the haematophagous ectoparasites in tree swallow nests, then I expected that parasite loads would be lower in broods where half the nestling received ivermectin injections (Launay and Leroy 1988, Levot and Sales 2002, 2008).

2.3 Methods

2.3.1 Study site, species, and general field procedures

I studied tree swallows, a species of aerial insectivore, breeding in nest boxes near Prince George, British Columbia, Canada (53°N, 122°W) in 2016 and 2017. The site had 139 nest boxes distributed along fence lines either in hay fields or beside roads bordering pastureland grazed by cattle. Fields were surrounded by second-growth forest. Nest boxes were mounted ~1.5 m above the ground on fence posts and nesting material was removed from all boxes at the end of each breeding season.

Ectoparasites parasitizing nestling tree swallows in this population include fleas, mites, and larval blow flies, though I only quantified the number of larval blow flies (*Protocalliphora* and *Trypocalliphora* spp.; Whitworth 2003) in nests because they typically are the most prevalent ectoparasite in this population. The life cycle, briefly, of blow flies, begins with adult

females laying eggs in the nest material of birds (Sabrosky et al. 1989). The resulting larvae spend the majority of their time in the nest material, but feed intermittently on the blood of nestlings by attaching externally. One exception is *T. braueri*, which embeds under the skin of nestlings to feed, often for the entire larval period. Larvae pupate and develop into adult flies following the third instar (Hall et al. 2017), overwinter, and reproduce the next summer (Sabrosky et al. 1989).

Beginning in early May, nest boxes were visited every two days to monitor nest-building, and daily once egg-laying began. Once egg-laying was complete, nests were not checked again until the predicted hatching date. Nests were then checked daily to determine the actual hatching date, which was designated as day 0 of the nestling period. Once all viable eggs had hatched in a nest, adults were trapped in nest boxes when feeding young, banded (if not previously banded), and measured. I used a spring scale (nearest 0.25 g) to measure body mass, a ruler (nearest 0.5 mm) to measure lengths of wing, ninth primary flight feather, tail and outer rectrix, and digital calipers (nearest 0.01 mm).to measure combined length of the head and bill ("head-bill"). These measurements were used to calculate the scaled mass index (SMI), as a proxy for adult condition (Peig and Green 2009), using the following equation:

$$\widehat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{b_{SMA}}$$

where M_i = body mass, L_i = the linear body measurement (here, length of head-bill because it was most strongly correlated with mass; females: r = 0.219, P = 0.010, males: r = 0.416, P < 0.0001), L_0 = the arithmetic mean of the linear body measurement for the population, and b_{SMA} = the slope from the ordinary least square regression between mass and length of head-bill, divided by the Pearson's r from the correlation between these two variables. The correlation and regression were both completed after the variables had been ln-transformed.

Nestlings were weighed two days post-hatch using a digital balance (nearest 0.01 g) and these measurements were used to calculate the degree of hatching asynchrony (hatching spread; the difference in mass between the smallest and largest nestlings within a brood at day 2, divided by mean mass of the brood) and relative nestling size within a brood (a nestling's day 2 mass minus the mean day 2 brood mass, divided by the standard deviation). Nestlings were then measured every two days from day 4 to 16 post-hatch. Body mass was measured using a spring scale (nearest 0.125 g), length of head-bill using digital calipers (nearest 0.01 mm), and length of ninth primary flight feather with a ruler (nearest 0.5 mm). Ninth primary feathers were not measured until 8 days post-hatch once the feathers had begun to grow. These measurements were used to generate growth rate constants using a logistic equation for mass, a Gompertz equation for head-bill, and a simple linear equation for ninth primary feather. Measurements taken at day 16 were used as a proxy for mass and size at fledging. On day 12 (2017) or 14 (2016), blood was collected (<100 µl) from the brachial vein by venipuncture. In 2016, blood was collected in a heparinized microcapillary tube, transferred to a microcentrifuge tube, and placed on ice for several hours before being frozen at -20°C until the haemoglobin concentration was determined (see below). In 2017, blood was collected in a cuvette and immediately inserted into a HemoCue® Haemoglobin Analyzer and the haemoglobin concentration determined. Nestlings were banded 16 days post-hatch and then left undisturbed (to reduce the chance of premature fledging) until 22 days when nests were checked daily to determine fledging success. Once a brood fledged, the nest was collected in a sealed bag to quantify the number of blow flies (see below).

2.3.2 Experimental design

Broods were paired by hatching date (± 1 day) and brood size (± 1 nestling), with one brood randomly assigned to the ivermectin (IVM) group and the other to the control group (2016: 32 brood pairs; 2017: 28 brood pairs). When nestlings were 6 days old, in IVM broods the nestlings were first ordered by mass and then every other nestling along the mass hierarchy received an injection of ivermectin (IVM), a broad spectrum, anti-parasite drug, alternating whether the heaviest nestling within a brood received IVM or oil. Ivomec® 1% Injection for Cattle and Swine was first diluted in sterile sesame oil to a concentration of 0.06 mg ivermectin/mL oil and then the appropriate volume was injected to achieve a dose of 0.2 mg/kg body weight. Nestlings in ivermectin broods that did not receive IVM injections and all nestlings in control broods received sham-injections of pure sterile sesame oil to a volume appropriate for their mass. Control injections ensured that any differences observed were not due to any possible negative effects of the injection or any possible positive effects of additional nutrients provided by the oil. Using a 0.3-mL syringe with a 29-gauge needle, nestlings were injected subcutaneously in the upper back (beside the spinal feather tract).

2.3.3 Nest dissections

Following collection, nests were left for at least 1 week at room temperature to allow any larval blow flies to pupate. Nests were then frozen for 24 hours and heated in an oven at 80°C for another 24 hours before being dissected by hand with the aid of sieves. The number of blow fly pupae and puparia was counted in each nest, and the total was considered to be the minimum parasite load for the nest.

2.3.4 Haemoglobin measurement

For whole blood samples in 2016, haemoglobin was measured using the cyanmethaemoglobin method of Drabkin and Austin (1935), modified for use with a plate reader. First, the Drabkin's solution was prepared by combining one vial of Drabkin's reagent (D5941, Sigma Aldrich Canada Inc.) with 1000 mL of water and 0.5 mL of 30% Brij L23 solution (B4184, Sigma Aldrich Canada Inc.). In each well, 1 µl of whole blood was thoroughly mixed with 250 µl of Drabkin's solution. Using a spectrophotometer plate reader (VersaMax Microplate Reader, Molecular Devices), the absorbance of each sample was read at 540 nm. The blood sample for each nestling was analyzed in duplicate and the absorbance averaged after the reading. The total haemoglobin concentration (mg/mL) was determined for each nestling using a calibration curve prepared with standards of known haemoglobin concentration (cyanmethaemoglobin standard: StanBio Laboratory, Texas, USA). Samples were measured in two assays with a mean intra-assay coefficient of variation (±SD) of 11.23 ± 0.38% and an inter-assay coefficient of variation of 15.87%. All values were lower than the coefficients of variation reported in a previous paper using the same method (Webster et al. 2015).

2.3.4 Statistical analyses

I used general mixed effects models (PROC MIXED; SAS Institute Inc., Cary, North Carolina, USA) to analyze the effect of the IVM treatment on nestling morphology and physiology. I examined the following response variables: mass and length of head-bill at day 6 (pre-injection); length of ninth primary feather at day 8 (2 days post-injection); mass, length of ninth primary feather and head-bill at day 16 (10 days post-injection); growth rates of mass, ninth primary feather and head-bill; and, haemoglobin concentration. As fixed effects, I included

IVM treatment (IVM brood/IVM injected, IVM brood/oil injected, control brood/oil injected), year, hatching date, brood size, relative brood asynchrony, relative nestling size within the brood, and length of ninth primary feather and condition (scaled mass index) of both parents. Adult variables were included as covariates because they indicate adult condition and quality, which can influence nestling phenotype (e.g., Laubach et al. 2015; Arai et al. 2018). Selected interactions were also included, which allowed me to examine if the effects of the treatment differed depending on the amount of morphological variation within a brood (treatment*relative brood asynchrony), a nestling's position within the brood size hierarchy (treatment*relative nestling size), and the timing of reproduction within the breeding season (treatment*hatching date). Because the breeding season was earlier in 2016 than 2017, hatching date was standardized within each year by calculating z-scores. Parasite load per nestling was not included in these analyses because treatment predicted a marginal amount of the variation in the number of parasites per nestling (see Results). Haemoglobin concentration was analyzed separately by year because different measuring techniques and sampling ages were used in 2016 and 2017. Nest identity was included in all models as a random effect (random intercept) to account for variation due to individual nests and clustered data effects. Prior to analysis, all fixed effects were tested for collinearity, but no variables had tolerance values less than 0.2.

To test for effect of the IVM treatment on the proportion of nestlings fledged, I used a generalized linear model (PROC GENMOD; SAS Institute Inc., Cary, North Carolina, USA) with a logit link and a binomial distribution. All the same variables were included as above, except for relative nestling size. Using a generalized linear mixed effects model (logit link and binomial family), I determined which variables predicted the chance of an individual nestling dying or successfully fledging. Again, the same variables as above were included as fixed effects

and nest ID was included as a random effect. Any nestlings that died during a two-day period of harsh weather in June 2016 (Chapter 4) or died prior to receiving an experimental injection (i.e., prior to day 6) were not included in the analysis.

Using analysis of covariance (ANCOVA; PROC GLM; SAS Institute Inc., Cary, North Carolina, USA), I investigated the effect of the IVM treatment on parasitism in nests by larval blow flies. As the response variable, I used the total number of pupae and puparia per nestling, which controlled for brood size. To meet the assumptions of normality and homogeneity of variance, this variable was square-root transformed prior to analysis. The IVM treatment at the brood level was included as the independent variable, where half the nestlings from IVM broods received IVM injections and all nestlings from control broods received control injections. I also included hatching date and year because the abundance of larval blow flies can vary predictably with the progression of the breeding season (e.g., increase: Pinkowski 1977; decrease: Hori and Iwasa 1988) and can show large fluctuations among years (e.g., Merino and Potti 1996).

For all analyses, I began with a full model that included all variables and interactions of interest. I then used a backward-stepwise procedure to eliminate any interactions and variables that did not approach significance ($P > 0.10$; Dupont 2009; Montgomery et al. 2012). Interactions were removed first, followed by single variables, in order of decreasing P-value. Results were considered significant at $P = 0.05$ level and marginal when $0.05 < P < 0.10$. Denominator degrees of freedom were calculated using Kenward-Roger's method for all mixed models (Kenward and Roger 1997). Means and parameter estimates are presented ± standard error. Sample sizes differ slightly among analyses because of missing data.

2.4 Results

On the day of experimental injections (day 6), mass and length of head-bill of nestlings did not differ among treatments, suggesting that no pre-manipulation biases existed among treatments (n = 65 broods, 297 nestlings; Table 2.1). Because ninth primary feathers had not emerged at the time of injection, feather measurements were instead compared two days post-injection (day 8), but also did not differ among treatments (n = 68 broods, 295 nestlings; Table 2.1).

IVM-injected nestlings were heavier than control nestlings at 16 days of age, regardless of whether control nestlings were from IVM or control broods, but this relationship only approached significance (P = 0.08, n = 65 broods, 297 nestlings; Table 2.2), and a significant interaction suggested that the effect of the IVM treatment on mass of nestlings depended on the relative brood asynchrony (P = 0.03). Mass of nestlings was positively related to length of ninth primary feather of female parents. When treatment levels were analyzed separately, mass was unrelated to relative brood asynchrony when nestlings were from IVM broods, regardless of whether the nestling received IVM-injections (estimate = -2.91 ± 2.11, $F_{1, 2.87} = 1.89$, P = 0.18, n = 34 broods, 79 nestlings) or oil-injections (estimate = 0.47 ± 1.65, $F_{1, 34.0} = 0.08$, P = 0.78, n = 35 broods, 81 nestlings), but showed a significant positive relationship when nestlings were from control broods (estimate = 4.39 ± 2.05, $F_{1, 26.7} = 4.57$, P = 0.04, n = 30 broods, 137 nestlings). IVM treatment had a marginal effect on length of ninth primary feathers at day 16 (P = 0.10), with nestlings from IVM broods having longer ninth primary feathers (marginally longer if injected with IVM (P = 0.09) and significantly longer if injected with oil (P = 0.04)) than nestlings from control broods (n = 68 broods, 296 nestlings; Fig. 2.1a). Length of ninth primaries was also longer in 2017 than 2016, positively related to relative nestling size within the brood

Table 2.1 Results of general mixed effects models testing whether nestling tree swallows (*Tachycineta bicolor*) differed morphologically by treatment level prior to (mass and length of head-bill) or two days after (length of ninth primary feather) the application of an anti-parasite treatment using ivermectin (IVM). In IVM broods, half of the nestlings received IVM injections and half received sham injections of sesame oil, whereas all nestlings received oil injections in control (ctl) broods.

Response Variable	Model Variables	Estimate	SE	df	t-value	P	95% CI	
							Lower	Upper
day 6 mass	IVM treatment	.	.	129.0	.	0.97	.	.
	brood size	-0.69	0.23	61.9	-2.99	<0.01	-1.15	-0.23
	relative nestling size	1.44	0.06	233.0	24.62	<0.0001	1.32	1.55
	female ninth	0.31	0.10	61.9	2.94	<0.01	0.10	0.52
day 8 length of ninth primary feather	IVM treatment	.	.	132.0	.	0.49	.	.
	brood size	-0.61	0.21	64.5	-2.92	<0.01	-1.02	-0.19
	relative nestling size	1.15	0.06	228.0	18.67	<0.0001	1.03	1.28
	male ninth	0.17	0.07	64.5	2.31	0.02	0.02	0.31
day 6 length of head-bill	IVM treatment	.	.	129.0	.	0.62	.	.
	relative nestling size	0.66	0.03	223.0	20.16	<0.0001	0.59	0.72
	female ninth	0.14	0.05	61.8	2.49	0.02	0.03	0.24
	brood size	-0.21	0.12	61.8	-1.70	0.09	-0.45	0.04

Table 2.2 Results of general mixed effects models examining the effect of an anti-parasite treatment (ivermectin; IVM) on the morphological traits of nestling tree swallows (*Tachycineta bicolor*) at 16 days of age. In control broods, all nestlings received sham injections of sesame oil (ctl-ctl), while in IVM broods, half of the nestlings received IVM injections (IVM-IVM) and half received control oil injections (IVM-ctl).

Response variable	Model variables	Estimate	SE	df	t-value	P	95% CI	
							Lower	Upper
day 16 mass	IVM treatment:	.	.	130.0	.	0.08	.	.
	IVM-IVM vs. ctl-ctl	2.63	1.41	71.6	1.87	0.07	-0.17	5.43
	IVM-ctl vs. ctl-ctl	1.51	1.38	67.7	1.09	0.28	-1.25	4.26
	IVM-IVM vs. IVM-ctl	1.12	0.64	237.0	1.76	0.08	-0.13	2.37
	relative brood asynchrony	-2.59	1.88	86.8	-1.38	0.17	-6.32	1.14
	female ninth	0.37	0.11	61.9	3.41	<0.01	0.16	0.59
	relative brood asynchrony*treatment:	.	.	130.0	.	0.03	.	.
day 16 length of ninth primary feather	IVM treatment:	.	.	129.0	.	0.105	.	.
	IVM-IVM vs. ctl-ctl	2.54	1.46	65.1	1.74	0.09	-0.37	5.46
	IVM-ctl vs. ctl-ctl	2.99	1.46	65.0	2.05	0.04	0.08	5.91
	IVM-IVM vs. IVM-ctl	-0.45	0.47	229.0	-0.96	0.34	-1.37	0.47
	year	3.46	1.47	61.9	2.35	0.02	0.52	6.40
	brood size	-1.25	0.73	62.2	-1.72	0.09	-2.70	0.20
	relative nestling size	2.76	0.20	227.0	14.05	<0.0001	2.38	3.15
	male ninth	0.65	0.26	61.8	2.50	0.02	0.13	1.18
day 16 length of head-bill	IVM treatment	.	.	137.0	.	0.196	.	.
	year	0.43	0.16	65.8	2.70	0.009	0.11	0.76
	relative brood asynchrony	-1.11	0.63	90.2	-1.77	0.080	-2.36	0.14
	relative nestling size	0.24	0.03	245.0	7.55	<0.0001	0.18	0.30
	relative brood asynchrony*treatment:	.	.	138.0	.	0.075	.	.

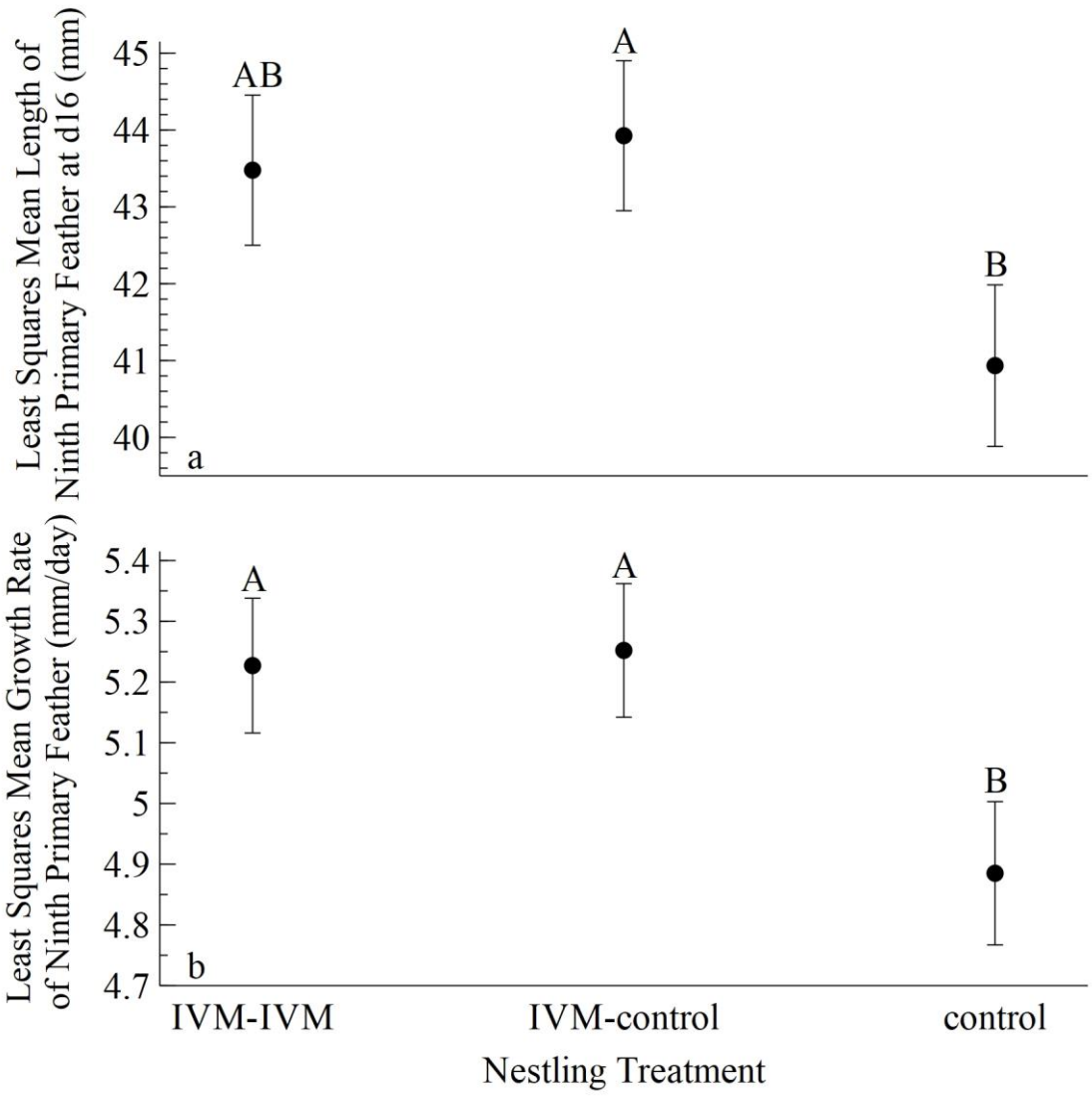

Figure 2.1 The least squares mean (± SE) of length (a) and growth rate (b) of ninth primary feathers of nestling tree swallows (*Tachycineta bicolor*) during an experiment where partial broods were treated with a broad spectrum, anti-parasite drug, ivermectin (IVM). In IVM broods, the length of ninth primary feather was marginally longer if nestlings received IVM injections (IVM-IVM; n = 37 broods, 85 nestlings) and significantly longer if nestlings received control injections of sesame oil (IVM-control; n = 37 broods, 81 nestlings) compared to nestlings from control broods, where all nestlings received oil injections (n = 31 broods, 130 nestlings). Similarly, the growth rate of ninth primary feather was significantly higher in IVM broods, regardless of whether nestlings received IVM injections (IVM-IVM; n = 36 broods, 84 nestlings) or control injections (IVM-control; n = 37 broods, 80 nestlings), than in control broods (n = 31 broods, 130 nestlings). Treatments that share the same letter were not significantly different ($P > 0.05$).

and length of ninth primary feathers of adult males, and negatively related to brood size (Table 2.2). Length of head-bill at day 16 also showed a year effect, with nestlings in 2017 having longer head-bill measurements than in 2016. Length of head-bill was positively related to relative nestling size within a brood (Table 2.2), and there was a non-significant interaction between IVM treatment and relative asynchrony of a brood on length of head-bill ($P = 0.08$, n = 72 broods, 319 nestlings). This interaction was likely the result of length of head-bill being positively related to relative hatching asynchrony in control nestlings from IVM broods (estimate = 0.66 ± 0.53, $F_{1, 37.5} = 1.54$, $P = 0.22$), but negatively related in IVM-injected nestlings from IVM broods (estimate = -0.24 ± 0.52, $F_{1, 33.8} = 0.21$, $P = 0.65$) and control nestlings from control broods (estimate = -0.52 ± 0.63, $F_{1, 28.3} = 0.69$, $P = 0.41$), although none of these relationships were significant.

Growth of mass was unrelated to the IVM treatment, but positively related to both relative nestling size within the brood and length of ninth primary feathers of adult males (n = 68 broods, 290 nestlings; Table 2.3). For growth rate of ninth primary feathers, nestlings from control broods had significantly slower feather growth than nestlings from IVM broods, regardless of whether nestlings received IVM or control injections, although the overall effect of treatment only approached significance ($P = 0.09$, n = 68 broods, 294 nestlings; Fig. 2.1b). Nestlings also had faster growing primaries in 2017 than 2016, when within a brood they were relatively larger, and if a male with longer ninth primary feathers raised them (Table 2.3). IVM treatment had no effect on growth rate of head-bill. Rather, the growth rate of head-bill was negatively related to brood size and positively related to length of ninth primary feathers of males (n = 68 broods, 298 nestlings; Table 2.3).

Table 2.3 Results of general mixed effects models examining the effect of an anti-parasite treatment (ivermectin; IVM) on growth rates of nestling tree swallows (*Tachycineta bicolor*). In control broods, all nestlings received sham injections of sesame oil (ctl-ctl), while in IVM broods, half of the nestlings received IVM injections (IVM-IVM) and half received control oil injections (IVM-ctl).

Response variable	Model variables	Estimate	SE	df	t-value	P	95% CI	
							Lower	Upper
mass growth rate	IVM treatment	.	.	127.0	.	0.80	.	.
	relative nestling size	0.01	0.01	222.0	2.11	0.04	0.00	0.02
	male ninth	0.02	0.01	61.5	3.34	<0.01	0.01	0.03
ninth growth rate	IVM treatment:	.	.	130.0	.	0.09	.	.
	IVM-IVM vs. ctl-ctl	0.34	0.17	66.2	2.08	0.04	0.01	0.67
	IVM-ctl vs. ctl-ctl	0.37	0.17	66.2	2.23	0.03	0.04	0.70
	IVM-IVM vs. IVM-ctl	-0.03	0.06	227.0	-0.45	0.65	-0.14	0.09
	year	0.42	0.17	62.9	2.55	0.01	0.09	0.75
	relative nestling size	0.21	0.02	226.0	8.87	<0.0001	0.16	0.25
	male ninth	0.07	0.03	62.4	2.25	0.03	0.01	0.13
head-bill growth rate	IVM treatment	.	.	125.0	.	0.65	.	.
	brood size	-0.01	0.01	61.0	-1.72	0.09	-0.02	0.00
	male ninth	0.01	0.00	60.8	3.23	<0.01	0.00	0.01

In 2016, haemoglobin concentration was not significantly affected by IVM treatment ($F_{2, 42.3} < 0.01$, $P > 0.99$) and none of the covariates explained a significant amount of the variation (n = 27 broods, 91 nestlings). In 2017, haemoglobin concentration differed by IVM treatment ($F_{2, 66.0} = 5.37$, $P = 0.01$, n = 42 broods, 123 nestlings; Fig. 2.2). Nestlings from IVM broods had higher haemoglobin concentrations than nestlings from control broods, regardless of whether nestlings from IVM broods received IVM (estimate = 15.27 ± 4.67, df = 42.3, P = 0.002) or control injections (estimate = 13.70 ± 4.69, df = 43.2, P = 0.006). Within IVM broods, however, control- and IVM-injected nestlings did not differ (estimate = 1.57 ± 2.73, df = 86.1, P = 0.57). Haemoglobin concentration in 2017 was also negatively related to hatching date (estimate = -7.00 ± 2.14, $F_{1, 35.9} = 10.68$, P = 0.002), relative brood asynchrony (estimate = -31.44 ± 14.20, $F_{1, 36.4} = 4.90$, P = 0.03) and length of ninth primary feather of adult males (estimate = -1.56 ± 0.89, $F_{1, 35.9} = 3.09$, P = 0.09), and positively related to brood size (estimate = 4.54 ± 2.58, $F_{1, 35.2} = 3.08$, P = 0.09) and relative nestling size within the brood (estimate = 3.67 ± 1.12, $F_{1, 85.1} = 10.67$, P = 0.002).

A marginally greater proportion of nestlings fledged from IVM broods (least squares mean (LSM) ± SE: 0.78 ± 0.04) than control broods (LSM ± SE: 0.66 ± 0.06; P = 0.096, n = 67 broods; Table 2.4). Proportion of nestlings fledged was significantly greater in 2017 broods than in 2016, and in broods where the adult male was in poorer body condition and had longer ninth primary feathers (Table 2.4). The probability of an individual nestling fledging increased with relative nestling size and decreased with condition of female parents, but there also was a marginally significant interaction between relative nestling size and IVM treatment (P = 0.06, n = 71 broods, 362 nestlings; Table 2.4). When treatment levels were analyzed separately to explore the interaction, relative nestling size showed a significant positive relationship with fate

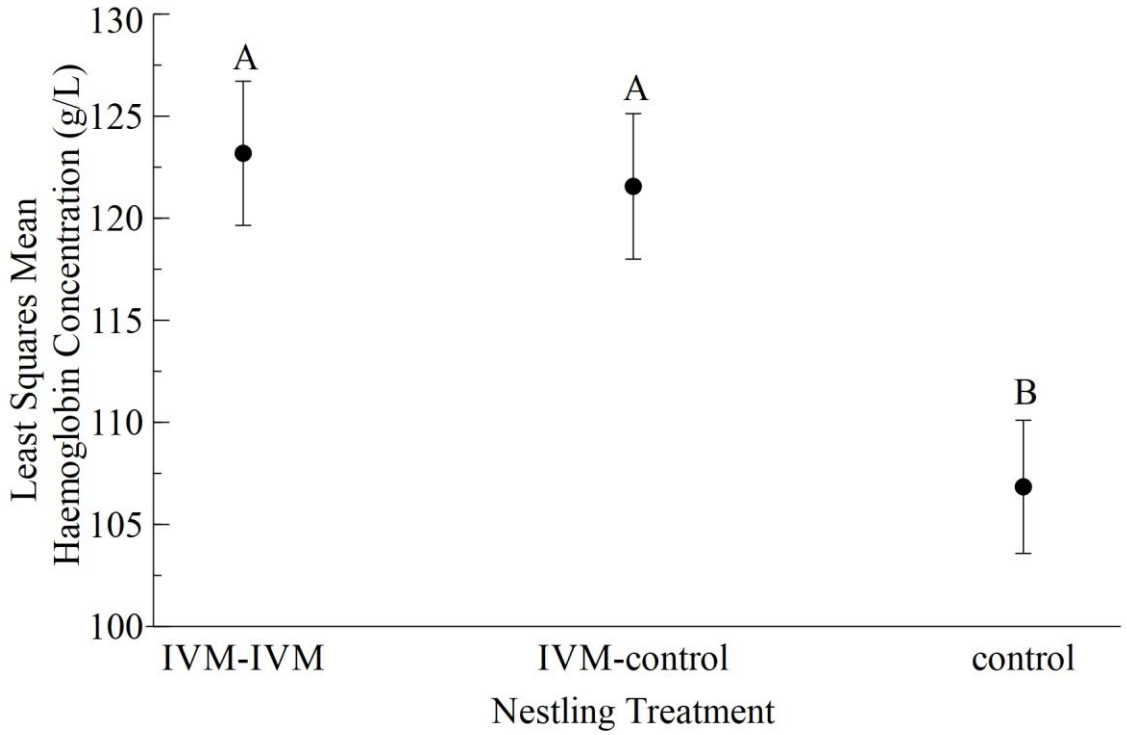

Figure 2.2 The least squares mean of haemoglobin concentration (± SE) of nestling tree swallows (*Tachycineta bicolor*) during one year (2017) of a two-year experiment using a broad spectrum, anti-parasite drug, ivermectin (IVM). The haemoglobin concentration was significantly higher in IVM broods, regardless of whether nestlings received IVM injections (IVM-IVM; n = 21 broods, 34 nestlings) or control injections of sesame oil (IVM-control; n = 21 broods, 30 nestlings), than in control broods, where all nestlings received oil injections (n = 21 broods, 59 nestlings). Treatments that share the same letter were not significantly different (P > 0.05).

Table 2.4 Results of a generalized linear model (proportion fledged) and a generalized linear mixed effects model (nestling fate) examining the effect of an anti-parasite treatment (ivermectin; IVM) on the fledging success of nestling tree swallows (*Tachycineta bicolor*). In control broods, all nestlings received sham injections of sesame oil (ctl-ctl), while in IVM broods, half of the nestlings received IVM injections (IVM-IVM) and half received control oil injections (IVM-ctl).

Response variable	Model variables	Estimate	SE	df	t-value	P	95% CI	
							Lower	Upper
proportion fledged	IVM treatment:	.	.	62	.	0.096	.	.
	IVM vs. ctl	0.70	0.41	.	1.69	0.096	-0.13	1.52
	year	0.94	0.41	62	2.28	0.03	0.12	1.76
	male condition	-0.40	0.17	62	-2.34	0.02	-0.75	-0.06
	male ninth	0.16	0.08	62	2.06	0.04	0.01	0.32
nestling fate	IVM treatment	.	.	188.1	.	0.15	.	.
	relative nestling size	1.19	0.38	355.0	3.18	<0.01	0.46	1.93
	female condition	-0.49	0.22	79.0	-2.28	0.03	-0.92	-0.06
	relative nestling size*treatment:	.	.	355.0	.	0.06	.	.

when nestlings were injected with IVM (estimate = 0.98 ± 0.36, $F_{1, 98.0} = 7.62$, P = 0.01, n = 38 broods, 101 nestlings), a marginally positive relationship when nestlings received control injections but had IVM-injected brood mates (estimate = 0.67 ± 0.37, $F_{1, 93.0} = 3.25$, P = 0.07, n = 38 broods, 96 nestlings), and no relationship when all nestlings in a brood received control injections (estimate = 0.18 ± 0.23, $F_{1, 162.0} = 0.59$, P = 0.44, n = 33 broods, 165 nestlings; Fig. 2.3).

Within years, the number of larval blow flies per nestling was negatively related with hatching date, such that later hatching nests had fewer larval blow flies and across years, the number of blow flies per nestling was significantly higher in 2016 than 2017 (Table 2.5). After controlling for these temporal effects, the number of larval blow flies per nestling was lower in nests where half of the nestlings received IVM-injections (LSM ± SE: 3.55 ± 4.84) than in nests where all nestlings received control injections (LSM ± SE: 4.84 ± 0.56), although the relationship only approached significance (P = 0.052, n = 72 broods; Table 2.5).

2.5 Discussion

Ivermectin (IVM) treatment positively affected nestling mass, growth of ninth primary feathers, haemoglobin concentration, and fledging success (Tables 2.2–2.4; Fig. 2.1–2.2), although the effect of the anti-parasite treatment on mass depended on the relative asynchrony within a brood. Nestlings from control broods tended to be heavier in more asynchronous broods, whereas mass of nestlings from IVM broods showed no relationship with asynchrony, regardless of whether they received oil or IVM injections. Because the IVM treatment reduced the abundance of larval blow flies within nests (Table 2.5), nestlings in control broods faced higher parasite loads. Therefore, these results suggest that in environments with higher parasite burdens,

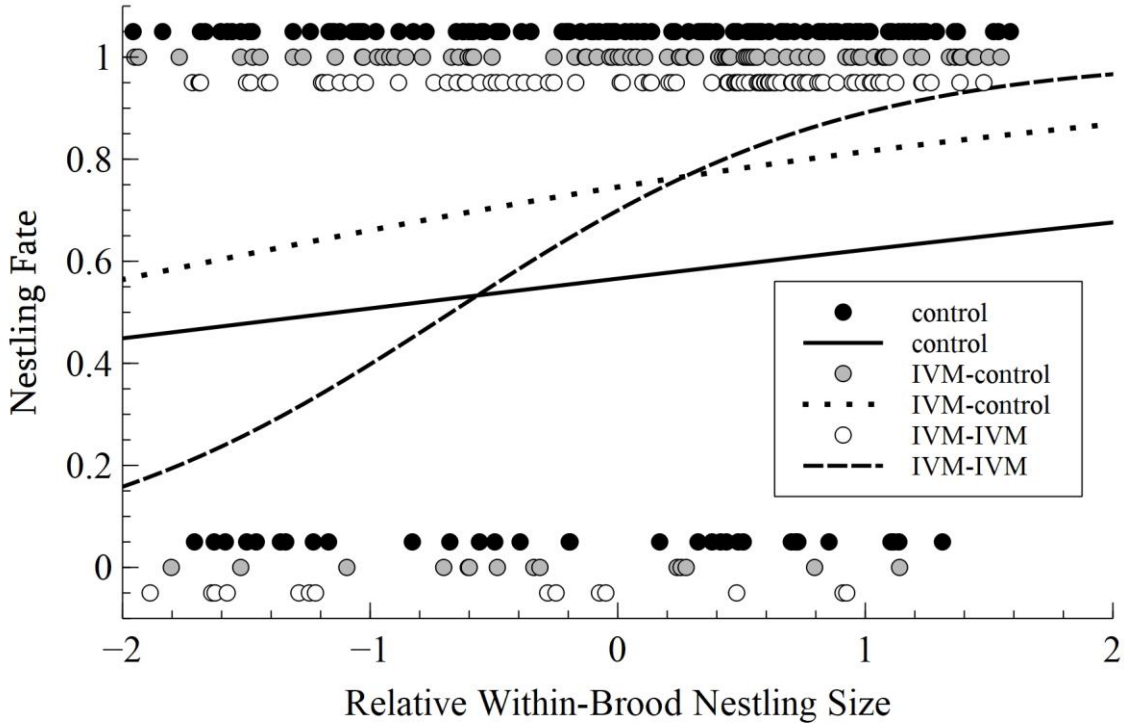

Figure 2.3 The effect of an anti-parasite drug treatment (ivermectin; IVM) on the fate of nestling tree swallows (*Tachycineta bicolor*) depended the on the relative size of a nestling within the brood. Fate was defined as 0 when a nestling died and 1 when a nestling fledged. Analysis by treatment level showed that nestlings from control broods (all nestlings received sham injections of pure sesame oil; solid line, black circles) had no relationship between fate and relative within-brood size, while nestlings from IVM broods, regardless of whether they received oil injections (dotted line, grey circles) or IVM injections (dashed line, white circles), had a positive relationship between fate and relative within-brood size, although this relationship was only marginally significant for oil-injected nestlings from IVM broods. Data points have been offset slightly from 0 and 1 by treatment to show their distribution.

Table 2.5 Results of an analysis of covariance examining the effect of an anti-parasite treatment (ivermectin; IVM) on the number of larval blow flies (*Protocalliphora* and *Trypocalliphora* spp.) per nestling found in nests of tree swallows (*Tachycineta bicolor*) over two years (2016 and 2017). In IVM broods, half of the nestlings received IVM injections and half received sham injections of sesame oil, whereas all nestlings received oil injections in control (ctl) broods.

Model Variables	Estimate	SE	df	t-value	P	95% CI	
						Lower	Upper
IVM treatment:							
IVM vs. ctl	-0.40	0.20	1	-1.98	0.05	-0.81	0.00
hatch date	-0.17	0.10	1	-1.67	0.01	-0.37	0.03
year	-1.05	0.21	1	-5.04	<0.0001	-1.47	-0.64

size hierarchies within broods can positively influence mass of nestlings, whereas in nests with lower parasite intensities, brood asynchrony had little effect on mass.

A review of 25 different bird species found that asynchronous broods generally produced nestlings with greater mean body mass than synchronous broods at the time of fledging, but fewer young per brood (Amundsen and Slagsvold 1991). Interestingly, my results agree with this general trend in mass, but only under high, not low, parasite loads. Nestling size is a strong predictor of post-fledging survival (reviewed in Naef-Daenzer and Grüebler 2016), and in tree swallows specifically, nestling body mass has been positively correlated with their probability of recruiting to the breeding population (Shutler et al. 2006; Harriman 2014). Therefore, why would brood asynchrony be beneficial (in this case, higher nestling body mass) at higher parasite loads, but not lower parasite loads? One possibility is that greater brood asynchrony allowed for brood reduction to occur more efficiently in parasite-rich environments (Slagsvold 1982, 1986, Gibbons 1987). The death of the smallest nestling within a brood may benefit the other brood mates when conditions are poor (the 'brood reduction hypothesis'; Lack 1947, Howe 1978) or under high parasite loads (the 'tasty chick hypothesis'; Christe et al. 1998). I did not find, however, that the smallest nestling was more likely to die in control broods (higher parasite loads), nor did I find that the degree of brood asynchrony predicted the proportion of nestlings fledged from a nest. It is possible that parasites aggregated on the smallest nestling ('tasty chick hypothesis'), which reduced the level of parasitism faced by other nest mates, but without increasing the chance of the smallest nestling dying. This would explain why I found no evidence for the smallest nestling having a lower chance of fledging, but why brood asynchrony was beneficial under higher parasite loads.

Hatching asynchrony could also be beneficial in high parasite environments if size hierarchies in asynchronous broods resulted in stable dominance hierarchies that reduced sibling conflict, and if less sibling conflict was particularly advantageous under higher parasite loads (the 'sibling rivalry reduction hypothesis'; Hamilton 1964, Hahn 1981, Mock and Ploger 1987). A less competitive environment also could be created in asynchronous broods if parents are better able to provide sufficient food when a hierarchy of size exists among nest mates. For example, in an asynchronous brood, the timing of when an individual nestling would require the most food (at the peak of its growth and development) may be staggered among nest mates, whereas in synchronous nests, all nest mates may reach the point of maximum food requirement at the same time, resulting in a greater burden on parents (the 'peak load hypothesis'; Hussell 1972). When parasite loads were low and parents did not have to compensate for the energy lost by nestlings to parasites, perhaps parents could provide sufficient food regardless of a brood's degree of asynchrony. In support, previous studies have shown that adult tree swallows can have flexible reproductive investment strategies (Shutler et al. 2006; Ardia 2007; Bortolotti et al. 2011). For example, in the context of declining seasonal patterns, adult tree swallows incurred the costs of deteriorating environmental conditions, rather than passing those costs onto their offspring (Bortolotti et al. 2011). Additional experiments would be required to tease apart the mechanism underlying this interaction, but it does illustrate the importance of considering the nest environment, such as parasite load, when investigating hatching asynchrony.

IVM treatment had no effect on the rate of growth of head-bill or mass but did affect the rate of growth of ninth primary feathers (Table 2.3). Nestlings from IVM broods grew their ninth primary feathers significantly faster than nestlings from control broods (Fig. 2.1b), suggesting higher loads of larval blow flies reduced growth of flight feathers. Because larval blow flies

consume the nestlings' blood, parasitized nestlings may have less energy to allocate to feather growth, resulting in their feathers growing more slowly. This slower growth appeared to result in shorter ninth primary feathers near to fledging for nestlings from control broods compared to nestlings from IVM broods (although control nestlings were only marginally different from IVM-injected nestlings, but significantly different from oil-injected nestlings from IVM broods; Table 2.2, Fig. 2.1a). Nestlings that face high parasite loads may increase feather growth, at a cost to body growth, to fledge sooner and reduce their exposure to blood-feeding, nest-dwelling ectoparasites. Nestling barn swallows (*Hirundo rustica*) facing experimentally elevated levels of ectoparasitic hippoboscid flies (Diptera) displayed faster growth of rectrix feathers than control nestlings, but slower growth of tarsi and mass (Saino et al. 1998). In addition, nestlings fledged sooner in response to high ectoparasite infestations (Møller 1990). In contrast, although I did not measure age at fledging, I found the opposite pattern, where control nestlings maintained mass and head-bill growth but not feather growth in response to higher parasite loads. Perhaps food resources were not abundant enough or parents could not provide a sufficient quantity or quality of food to allow for rapid feather growth. Longer rearing periods in parasitized broods compared to broods where parasites were removed have been previously documented in alpine swifts (*Apus melba*; Bize et al. 2004), but growth rates were not measured, so it is impossible to say whether the longer rearing period was a result of slower nestling growth.

Alternatively, the inability of more heavily parasitized nestlings to attain similar feather growth as less heavily parasitized nestlings may be explained by the dominant biomolecules used in different morphological components. The development of feathers, which are composed of keratin, would be limited by the availability of protein, whereas mass may be influenced by fat intake and thus, the quality and quantity of the food adults provide to nestlings (e.g., Twining et

al. 2016). Because haematophagous ectoparasites, such as larval blow flies, can reduce haemoglobin concentration (Minias 2015), parasitized nestlings may have less protein available to invest in feather growth if they are regenerating haemoglobin, a protein molecule. Length of head-bill, a measure that incorporates skull size and thus, reflects skeletal size, may be more influenced by genetics (Wiggins 1989, 1990) or the procurement of calcium (e.g., Dawson and Bidwell 2005).

In 2017, when a larger sample size was used to measure haemoglobin concentration (42 broods in 2017 vs. 27 broods in 2016), an effect of IVM treatment was found, where haemoglobin concentration of nestlings from control (more heavily parasitized) broods was lower than nestlings from IVM (less parasitized) broods (Fig. 2.2). This agrees with many previous studies that have found a negative relationship between the intensity of infestation by haematophagous parasitism and haemoglobin concentration (reviewed in Minias 2015). Haemoglobin concentration has been positively associated with a number of nestling characteristics, including quality of diet (Pryke et al. 2011, Pryke and Rollins 2012), size-corrected body mass (O'Dwyer et al. 2007, Lill et al. 2013), and brood size (Minias et al. 2015), suggesting that lower haemoglobin concentrations may be costly during the nestling phase. Because these previous studies were correlational, it is not known whether haemoglobin concentration directly affected nestling morphology, or if nestling morphology is influenced by other factors that also co-vary with haemoglobin concentrations. If haemoglobin concentration does influence nestling morphology, then the reduction in nestling haemoglobin concentration by haematophagous ectoparasites may have been the underlying mechanism causing the negative morphological effects I found on nestlings from control broods relative to those from IVM broods. Because lower haemoglobin concentration reduces the oxygen-carrying capacity in birds

(Minias 2015), reduced haemoglobin levels could directly affect growth and development if less oxygen is available for these processes or indirectly by reducing begging behaviour. When begging, nestling tree swallows use significantly more oxygen (an estimated 28% more energy) than resting at both 5 and 10 days of age (Leech and Leonard 1996) and thus, parasitized nestlings with lower haemoglobin concentrations may beg at lower rates. This could lead to those nestlings receiving less food from parents (Leonard and Horn 1996) and could help explain why I found marginally lower mass and shorter ninth primary feathers, slower growth rates of primary feathers and decreased fledging success in control broods compared to IVM broods.

IVM treatment affected fledging success, with a marginally higher proportion of nestlings fledging from IVM broods than control broods (Table 2.4). High parasite loads have previously been associated with lower fledging success in some species (Møller 1990, Bańbura et al. 2004, Koop et al. 2011, Pryor and Casto 2017). Nestlings in IVM broods were exposed to lower parasite loads and as a result, may have had more resources available to invest in their own growth and development, as well as survival. The faster growth and longer length near to fledging of flight feathers, and higher haemoglobin concentrations, of nestlings from IVM broods may have improved their chance of fledging. The reduced oxygen-carrying capacity of nestlings with lower haemoglobin concentrations can decrease fledgling survival during the more active post-fledging period (Clayton and Tompkins 1994, Streby et al. 2009), but could also reduce the ability of nestlings to initially leave the nest, particularly if parents were unable to compensate for the costs of parasitism. In addition, the chance of individual nestlings fledging increased with relative nestling size in IVM broods (perhaps small nestlings were less likely to fledge because they have fewer reserves to survive food shortages (e.g., Gibbons 1987) or tolerate cold weather conditions (Boyle et al. 2013; Chapter 4)), but was unrelated to relative

nestling size in control broods (Table 2.4; Fig. 2.3). Under higher parasite loads (control broods), larger nestlings within a brood had a reduced chance of surviving relative to nestlings from broods with lower parasite loads (IVM broods), while smaller-sized nestlings within a brood had increased chances of surviving. This suggests that either 1) the virulence of larval blow flies is greater for larger nestlings, perhaps because larger nestlings invest more resources in resisting parasitism (e.g., via immunological responses) and therefore, have fewer resources available for their own development and survival, or because larger nestlings are less tolerant of parasitism, where tolerance is the ability to reduce damage caused by parasites without decreasing parasite fitness, and therefore, suffer a greater cost compared to smaller nest mates (e.g., DeSimone et al. 2018), or 2) parasites preferentially feed on larger nestlings, resulting in larger nestlings paying a greater cost of parasitism relative to their smaller nest mates. Either of these two possibilities may reduce the competitive pressures faced by smaller nestlings within a brood, increasing their chance of survival when parasite loads are higher. Alternatively, if parasites preferentially feed on the smallest nestling within a brood (i.e., the 'tasty chick hypothesis'), then perhaps larger nestlings may be less able to cope with ectoparasites. Under higher parasite loads, larval blow flies may feed on larger nestlings within a brood more than at lower parasite loads due to a limited number of feeding sites on the smallest nestling. Thus, at higher parasite loads (i.e., control broods), larger nestlings may be fed on more frequently and because they are less tolerant of parasitism or invest more resources in resisting parasitism, suffer greater costs than smaller nestlings facing the same parasite load. The first step in teasing apart this relationship would be to determine if larval blow flies preferentially feed on certain nestlings within broods of tree swallows.

The number of larval blow flies was marginally lower in nests where partial broods were injected with IVM than in nests where full broods were injected with oil (Table 2.5). Ectoparasites that feed on the blood of IVM-injected nestlings are expected to suffer negative consequences, including death (Launay and Leroy 1988, Levot and Sales 2002, 2008). Therefore, enough ectoparasites may have died from feeding on IVM-injected nestlings to cause the marginal reduction in parasite load in these nests. Alternatively, the ivermectin may have had less severe effects and simply reduced blood meal size or number of blood meals of larval blow flies. This could lead to larvae being too small to undergo successful pupation (Bennett and Whitworth 1991), which would result in larvae decaying and going undetected at the time of nest dissections. Although I cannot confirm the mechanism, the anti-parasite treatment, which was applied to individual nestlings, did marginally reduce the parasite load of nests and, given my other results, appeared to benefit the brood as a whole (i.e., both control- and IVM-injected nestlings within IVM broods).

In conclusion, I found that an anti-parasite treatment that decreased the parasite load of a nest positively affected nestling morphology (length at 16 days of age and growth rate of ninth primary feather), physiology (haemoglobin concentration), and fledging success, regardless of whether nestlings directly received the anti-parasite treatment themselves or had nest mates that had received it. I only observed within-brood differences in nestling response to the anti-parasite treatment in terms of the probability of individual fledging success, suggesting that larger nestlings within broods may either be more sensitive to the negative effects of parasitism or parasites may preferentially feed on larger-sized nestlings at higher parasite loads. Thus, my study has experimentally demonstrated the importance of nestling susceptibility to parasites at both the brood- and nestling-level. Future studies should explore the mechanism causing larger-

sized nestlings to have lower survival relative to smaller-sized nestlings when parasite loads are higher, possibly by first identifying whether larval blow flies preferentially feed on certain nestlings within broods of tree swallows.

3. Experimental reduction of nestling haemoglobin concentration and nest ectoparasite load affects nestling morphology, but not the behaviour of nestling or adult tree swallows (*Tachycineta bicolor*)

3.1 Abstract

Young, nest-bound birds often face a diversity of ectoparasites that typically feed on blood. These parasites can negatively affect a wide range of nestling morphological traits, as well as physiological variables, such as haemoglobin concentration. While haemoglobin concentration is often correlated with nestling morphology, it is unknown whether variation in haemoglobin is the direct proximate factor responsible for variation in morphology, or if nestling traits simply co-vary with haemoglobin concentration due to a number of similar factors influencing both. If haemoglobin concentration does directly influence the phenotype of nestlings, then the reduction in nestling haemoglobin concentration by ectoparasites may cause some other negative effects commonly associated with parasitism (e.g., slowed morphological development and reduced fledging success). By experimentally reducing haemoglobin concentration of nestling tree swallows (*Tachycineta bicolor*) using phenylhydrazine hydrochloride (PHZ), my objective was to test whether reduced haemoglobin concentration could be the mechanism that causes changes in nestling morphology, behaviour, and physiology in response to increased parasitism. Parasite loads were manipulated in conjunction with the PHZ treatment. Nestlings injected with PHZ displayed no change in haemoglobin concentration two days post-injection, whereas control nestlings showed a typical increase. By six days post-injection, PHZ-injected nestlings had recovered to normal haemoglobin levels and only the parasite treatment affected haemoglobin concentration. Both treatments affected growth and length of flight feathers, while only the

parasite reduction treatment affected fledging success. I have not only shown that both parasites and haemoglobin concentration can influence nestling morphology, but also demonstrated that PHZ can successfully be used in wild, nestling birds to experimentally investigate the effects of reduced haemoglobin concentration. My validation of this technique may prompt more experiments in a diversity of species.

3.2 Introduction

Young, altricial birds often are infested by various haematophagous, nest-dwelling ectoparasites during the brood-rearing stage, such as mites, fleas, and larval blow flies. Though the presence of these parasites may promote the development of the immune system and thereby provide some benefit, ectoparasites have a number of negative impacts on nestlings. Blood-feeding parasites can affect nestling growth (lighter mass at fledging: Pryor and Casto 2017), physiology (lower haemoglobin concentration: Minias 2015; decreased plasma protein content: Saino et al. 1998), and fledging success (Richner et al. 1993). The severity of these effects can depend on the intensity of parasite infestation (McKilligan 1996), the ability of parents to compensate for the energy nestlings lose to parasites (Christe et al. 1995), and environmental conditions (Dufva and Allander 1996).

Reduced haemoglobin concentration, one commonly documented negative effect of haematophagous ectoparasites, results in decreased oxygen carrying capacity for nestlings. This can have obvious implications for young birds once they fledge and are more aerobically active. For example, fledgling ovenbirds (*Seiurus aurocapillus*) that were heavily parasitized by larval blow flies during the nestling stage had lower survival and shorter initial flight distances than fledglings from non-infested nests (Streby et al. 2009). While aerobically less active, young nest-

bound birds may also face negative consequences of reduced haemoglobin concentrations, as a lower oxygen carrying capacity may be detrimental for activities that require oxygen such as begging, growth, and metabolism.

Nestling begging is expected to be costly, according to both the theory of parent-offspring conflict (Trivers 1974) and honest signaling (Grafen 1990; Godfray 1991). One cost could be the attraction of predators (e.g., Leech and Leonard 1997), but begging can also be costly due to the energy required to perform this behaviour. For example, tree swallows (*Tachycineta bicolor*) use significantly more oxygen (an estimated 28% more energy) when begging than resting at both 5 and 10 days of age (Leech and Leonard 1996). Similarly, McCarty (1996) found that the metabolic rate of nestling tree swallows when begging was 1.27 times the resting metabolic rate and it cost 0.008 J/g to beg for one second. While this cost increased with age, it was relatively low when compared with the energetic costs associated with other avian behaviours (McCarty 1996), and growth of nestling tree swallows was not affected by begging behaviour (Leonard et al. 2003).

As growth and metabolic processes require high levels of energy and oxygen (Buyse and Decuypere 2015; Scanes 2015a, b), the oxygen carrying capacity of a nestling, measured as haemoglobin concentration, may limit growth. Haemoglobin concentration was positively associated with size-corrected body mass in nestling Gould's petrels (*Pterodroma leucoptera*; O'Dwyer et al. 2007), and body mass and four different indices of size-corrected body mass in nestling welcome swallows (*Hirundo neoxena*; Lill et al. 2013). In tree swallows, haemoglobin concentration was positively related to the relative size of nestlings within a brood (Chapter 2).

While haemoglobin concentration has been correlated with a number of other nestling traits, like morphology (Lill et al. 2013) and fledging success (Kaliński et al. 2017), parasitism

also can affect a wide range of nestling traits, both morphological and physiological (Pryor and Casto 2017), including haemoglobin concentration (DeSimone et al. 2018). It is unknown, however, whether variation in haemoglobin could be the direct proximate factor responsible for variation in morphology, or if nestling traits simply co-vary with haemoglobin concentration due to a number of similar factors influencing both (e.g., quality of diet: Pryke et al. 2011). If haemoglobin concentration does directly influence the phenotype of nestlings, then the reduction in nestling haemoglobin concentration by ectoparasites may cause some of the other negative effects commonly associated with parasitism. By experimentally reducing haemoglobin concentration of nestling tree swallows using phenylhydrazine hydrochloride (PHZ), my objective was to test whether reduced haemoglobin concentration could be the mechanism that causes changes in nestling morphology, behaviour, and physiology in response to increased parasitism. Parasite loads were manipulated (using a nest-heating treatment) in conjunction with the PHZ treatment in a 2x2 design. If a nestling's growth, development, and behaviour were limited by its oxygen carrying capacity, then nestlings with lower haemoglobin concentrations (i.e., PHZ-injected nestlings and nestlings in parasite-infested environments) would be negatively affected by the treatment compared to nestlings with higher haemoglobin concentrations (i.e., sham-injected nestlings and nestlings in parasite-free environments). Specifically, I predicted that nestlings treated with PHZ and reared in parasite-infested environments would be morphologically smaller, have slower rates of growth, greater concentrations of plasma β-hydroxybutyrate (a ketone body that acts as a circulating energy source for tissues during times of fasting; Newman and Verdin 2014), less vigorous begging behaviour, and lower fledging success. Furthermore, if certain nestlings within the size hierarchy of a brood are more susceptible to parasitism because they are less tolerant of reductions in

haemoglobin (e.g., Christe et al. 1996a), then those nestlings would be more affected by the PHZ treatment. Lastly, as parents may amelioriate the negative effects of parasites on nestlings by responding behaviourally (e.g., Christe et al. 1996b; Bouslama et al. 2002), I tested whether adults increased feeding rate or saniation behaviour when their young faced higher parasite loads or a reduction in haemoglobin concentration.

3.3 Methods

3.3.1 Study species and area

Between May and August 2017, I studied the tree swallow, an aerial insectivorous bird, near Prince George, British Columbia, Canada (53°N, 122°W). Tree swallows are secondary cavity nesters and readily accept nest boxes. The field site consisted of a series of managed wetlands, surrounded by second-growth forest. Sixty nest boxes were placed around the edges of the ponds and mounted ~1.5 m above the ground on metal poles or wooden fence posts. Nest material was cleaned out at the end of each breeding season.

In this population of tree swallows, nestlings are commonly parasitized by fleas, mites, and larval blow flies, although only the number of larval blow flies (*Protocalliphora* and *Trypocalliphora* spp.; Whitworth 2003) in nests was quantified for this study. Adult female blow flies lay eggs in the nests of birds and upon hatching, the larvae spend the majority of their time in the nest material, feeding intermittently on the blood of nestlings (Sabrosky et al. 1989). The one exception, *T. braueri,* embeds under the skin of nestlings to feed and often remains embedded for the entire larval period (Sabrosky et al. 1989). Following three instars, larvae pupate, develop into adult flies and, upon emerging, overwinter and reproduce the next summer (Sabrosky et al. 1989).

3.3.2 General field methods

Beginning in May, nests were checked every other day to monitor nest development. Nests were checked daily once the birds began laying eggs to determine the start of incubation, at which time nest monitoring was temporarily suspended. On the predicted hatching date, monitoring was resumed to determine the actual hatching date, which was considered day 0 of the nestling period. After all viable eggs had hatched, adults were captured in the nest box while feeding offspring, banded (if not previously banded), and measured. A spring scale was used to measure body mass (nearest 0.25 g), a ruler was used to measure length of the wing, ninth primary flight feather, tail and outer rectrix (nearest 0.5 mm), and digital calipers were used to measure length of combined head and bill (hereafter 'head-bill'; nearest 0.01 mm). These measurements were used to calculate the scaled mass index, as a proxy for adult condition (Peig and Green 2009), using the following equation:

$$\widehat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{b_{SMA}}$$

where M_i = body mass, L_i = the linear body measurement (here, length of head-bill for males (r = 0.351, P = 0.002) and length of wing (r = 0.345, P = 0.002) for females, as each were most strongly correlated with mass), L_0 = the arithmetic mean of the linear body measurement for the population, and b_{SMA} = the slope from the ordinary least-square regression divided by the Pearson's r from the correlation between these two variables. The correlation and regression were both fit after the variables had been ln-transformed.

On day 2, nestlings were weighed using an electronic balance (nearest 0.01 g), and these data were used to estimate the degree of hatching asynchrony (hatching spread; the difference in mass between the smallest and largest nestlings within a brood at day 2, divided by mean mass of the brood) and relative nestling size within a brood (a nestling's day 2 mass minus the mean day

2 brood mass, divided by the standard deviation). Every two days between day 4 and 16, mass was measured using a spring scale (nearest 0.125 g), length of the ninth primary flight feather was measured using a ruler (nearest 0.5 mm), and length of head-bill was measured using digital calipers (nearest 0.01 mm). Ninth primary feather measurements did not begin until day 8, once the primary feathers had emerged. These measurements were used to calculate growth rate constants for each nestling, using a logistic equation for mass, a linear equation for ninth primary feather, and a Gompertz equation for head-bill. Morphological measurements at day 16 were used as a proxy for nestling size near to fledging. Blood samples were collected on day 12 from the brachial vein by venipuncture. Ten µl were collected in a cuvette and immediately inserted into a HemoCue® haemoglobin analyzer (HemoCue AB, Ängelholm, Sweden), which measured haemoglobin concentration. Another 15 µl of blood were collected in a test strip, and inserted into a Precision Xtra® ketone meter (Abbott, Abbott Park, IL), which measured β-hydroxybutyrate concentration. Nestlings were banded on day 16 and then left undisturbed (to reduce the risk of premature fledging) until day 22, when I determined fledging success. Nests were collected in individual sealed plastic bags once a brood fledged to quantify the number of blow flies (see below).

Throughout the nestling rearing stage (early June to end of July), a weather station located on site was programmed to collect data every minute on the amount of rainfall (mm), wind and gust speed (m/s), wind direction, temperature (°C), and relative humidity (%).

3.3.3 Experimental procedure

Beginning on day 4 and continuing every two days until day 16, at half of the nests nestlings were temporarily removed and nests placed in a sealed plastic bag and heated in a

generator-powered microwave oven to kill all nest-dwelling parasites. Cardboard inserts in nest boxes allowed for easy removal of nests with minimum disturbance. Control nests were also removed from the nest box every other day, but were not heated in a microwave. In a 2x2 design, the heating treatment was paired with a treatment experimentally reducing haemoglobin concentration. On day 6, pairs of nests were matched for hatching date (± 1 day) and brood size (± 1 nestling), and randomly assigned to treatment and control broods. In treatment broods, all nestlings received an injection of phenylhydrazine hydrochloride (PHZ), a chemical that reduces haemoglobin concentration by lysing red blood cells (Williams et al. 2012). PHZ was either diluted in saline to a concentration of 10 mg PHZ/mL saline or 11.8 mg PHZ/mL saline, and the appropriate volume was injected to achieve a dose of either 12.5 μg PHZ/g body mass (dose validated in Williams et al. 2012 in adult Zebra Finches (*Taenipoygia guttata*) and European Starlings (*Sturnus vulgaris*)) or 15.0 μg PHZ/g body mass. The higher dose was also tested as nestlings were expected to recover more quickly than adults from the effects of the chemical. In control broods, all nestlings received sham-injections of saline to a volume appropriate for their mass to ensure that any differences observed were not due to any possible negative effects of the injection. All injections (control or PHZ) were intramuscular and performed using a 0.3-mL syringe with a 29-gauge needle. The 2x2 design resulted in four treatment combinations (heated nest/PHZ brood, heated nest/control brood, control nest/PHZ brood, control nest/control brood). To confirm that the PHZ successfully reduced haemoglobin concentration, blood was collected from 20 nestlings from treatment broods (10 nestlings injected with the 12.5 μg/g dose and 10 nestlings injected with the 15.0 μg/g dose) and 20 nestlings from control broods on both day 6 and 8, and the haemoglobin concentration determined for these repeated samples.

In the morning on day 8, I attached a video camera (HawkEye HD Nature Camera, Birdshouse Spy Cam, West Linn, OR) to the inside of the roof of the nest box to quantify provisioning and begging behaviour. The camera was connected to a digital video recorder and battery and was left to record until late in the afternoon, at which time the camera equipment was removed. The head of each nestling was uniquely marked using non-toxic paint prior to recording so that nestlings could be individually identified during video analysis.

3.3.4 Nest dissections

Following collection, nests were left in sealed plastic bags at room temperature for at least a week to allow all larval blow flies to pupate. Nests were then placed in a freezer for 24 hours and then heated in an oven at 80°C for 24 hours. Nests were dissected and all blow fly pupae and puparia were counted. The total sum of pupae and puparia, which represented the total parasite load of a nest, was divided by the brood size to obtain the number of larval blow flies per nestling.

3.3.5 Video analysis

For each video recording, the first 30 minutes was discarded to ensure the adults had habituated to the camera. For the next three hours of the observation period, the following data were recorded: time of adult arrival and departure, whether a nestling was fed, the number of times an adult tremble-thrusted during a visit (adult dives head-first into nesting material and pecks throughout, while moving wings vigorously; see Pinkowski 1976) and the total time spent tremble-thrusting during a visit. For the first two hours of observation, begging behaviour was recorded for each nestling. Specifically, begging duration and begging score of both primary

(adult present in nest box) and secondary (adult absent) begging were recorded. For begging score, I followed Leonard et al. (2003), where begging vigour increases on a 5-point scale: 1 (head down, gaping, sitting on tarsi), 2 (head up), 3 (neck stretched upward), 4 (body lifted off tarsi), and 5 (wings waving). The average of the begging duration and score were multiplied to obtain a measure of mean begging intensity for each nestling.

3.3.2.3 Statistical analyses

As environmental variables are known to influence nestling morphology (e.g., Ambrosini et al. 2006) and physiology (e.g., Markowski et al. 2015), including haemoglobin concentration (Kaliński et al. 2009), weather variables were included in analyses. I first reduced the hourly measurements of ambient temperature (°C), rainfall (mm), and wind speed (ms^{-1}) into a single daily weather index, following Pelayo and Clark (2003) and modifications by Harriman et al. (2017). Briefly, I calculated the standardized values (z-scores) for total rainfall, mean and maximum wind speed, and mean and minimum temperature. These values were summed together such that more negative values indicated cooler, windier, and rainier conditions. I then followed the procedure outlined in Ambrosini et al. (2006) to select the best time period prior to the date of measurement for each morphological and physiological trait for which to examine effects of weather. This entailed that for each response variable, I ran a series of mixed effects models that included the daily weather index for each day, ranging from the day of measurement to 12 days prior to measurement. Models also included nest ID as a random effect and treatment, clutch initiation date (CID) and brood size as fixed effects. For each time interval, I calculated the Wald Z (the parameter estimate divided by its asymptotic standard error) for the daily weather index of the single day prior to measurement, as well as the average daily weather index

for all days within the time interval. By comparing the Wald Z values, I selected the time interval with the maximum predictive capability to use in the analyses. For mass, length of ninth primary feather, and length of head-bill at day 16, I used the daily weather index two, nine, and two days prior to the date of measurement, respectively. For haemoglobin concentration at day 12, I used the daily weather index three days prior to measurement, while the daily weather index on the day of measurement was the best choice for β-hydroxybutyrate concentration. For growth rates that were calculated over a 12-day period, I averaged the daily weather index values for all 12 days. Similarly, for all behavioural data obtained from video recordings, I calculated weather indices for the exact time period that the observations were made.

To test the effects of the two treatments, nest heating and PHZ injections, I used general mixed effects models (PROC MIXED; SAS Institute Inc., Cary, NC) for all nestling-level analyses, and analysis of covariance (PROC GLM) for all brood-level analyses. Two exceptions were the proportion of nestlings fledged from a brood, in which I used a generalized linear model with a logit link and a binomial distribution, and the number of larval blow flies per nestling, in which I used a Mann-Whitney U test because the assumptions of normality and homogeneity of variance were not met. As response variables, I examined the following at the nestling-level: mass, length of head-bill and haemoglobin concentration at day 6 (pre-injection); length of ninth primary feather at day 8 (two days post-injection); mass, length of ninth primary feather and head-bill at day 16 (10 days post-injection); growth rate of mass, ninth primary feather and head-bill; the within-individual change in haemoglobin concentration between day 6 and 8, haemoglobin concentration at day 8, haemoglobin and β-hydroxybutyrate concentrations at day 12, and mean primary and secondary begging intensity. For change in haemoglobin

concentration between day 6 and 8, I used a repeated measures mixed effects model with nestling age included as the repeated measure.

At the brood-level, I examined the following response variables: mean rate of per capita feeding (feeding rate divided by brood size), tremble-thrusting and sanitation duration, and the proportion of young fledged from a brood. I included the following fixed effects: PHZ by heat treatment (four treatment levels: heat-PHZ, heat-control, control-PHZ, control-control), hatching date, weather index, brood size, relative brood asynchrony, relative nestling size within the brood (nestling-level analyses only), length of ninth primary feather and condition of both adults (not included in feeding and sanitation models because sex of adult could not be determined in videos), and selected interactions (treatment*hatching date, treatment*relative brood asynchrony, treatment*relative nestling size). As a random effect (random intercept), I included nest identity in all nestling-level models, which accounted for clustered data effects and variation due to individual nests. In all mixed models, denominator degrees of freedom were calculated using Kenward-Roger's method (Kenward and Roger 1997). Fixed effects were tested for collinearity before performing analyses and no variables had tolerance values less than 0.2.

For all analyses, I used a backward-stepwise procedure, beginning with a full model that included all variables and interactions of interest, and then removed any terms (interactions first, followed by variables) that did not approach significance ($P > 0.10$; Dupont 2009; Montgomery et al. 2012).

3.4 Results

On day 6 when experimental injections were given, nestlings did not differ in mass, length of head-bill or haemoglobin concentration among treatments, nor did length of ninth

primary feather differ two days post-injection (day eight), suggesting that randomization of treatment assignment was successful (Table 3.1). Ninth primary feathers could not be compared on the day of injection because the feathers typically do not emerge until day 8.

Among individuals, haemoglobin increased from day 6 to 8 (n = 78 nestlings, 12 broods; Table 3.2). The interaction between treatment and age was significant, however, suggesting that the relationship between change in haemoglobin concentration and age depended on treatment (Fig. 3.1). When analyzed separately by treatment combination, haemoglobin concentration increased significantly with age for nestlings in heated nests receiving control injections (estimate = 7.50 ± 1.85, $F_{1,23.4} = 16.41$, $P < 0.001$) and marginally so for nestlings in parasitized nests receiving control injections (estimate = 5.25 ± 2.55, $F_{1,8.69} = 4.24$, $P = 0.07$), while no relationship existed when nestlings received PHZ-injections, regardless of whether their nest was heated (estimate = 0.27 ± 1.95, $F_{1,17.9} = 0.02$, $P = 0.89$) or not (estimate = -0.44 ± 2.17, $F_{1,9.89} = 0.04$, $P = 0.84$). When nestling haemoglobin concentration at day 8 was analyzed, rather than the change from day 6 to 8, treatment had a significant effect overall (Table 3.2). Specifically, control-injected nestlings from heated nests had significantly greater haemoglobin concentrations than control-injected nestlings from parasitized nests and PHZ-injected nestlings from both parasitized and heated nests, while all other comparisons among treatments were not significantly different (Table 3.2, Fig. 3.1). At day 12 (six days post-injection), haemoglobin concentration was still influenced by treatment, but nestlings raised in parasitized nests, regardless of whether they received control or PHZ injections, had significantly lower haemoglobin concentrations than nestlings raised in heated nests, again regardless of the type of injection they received (n = 77 nestlings, 26 broods; Fig. 3.1). Haemoglobin concentration at day

Table 3.1 Results of general mixed effects models testing whether the morphology or physiology of nestling tree swallows (*Tachycineta bicolor*) differed prior to (mass, length of head-bill and haemoglobin concentration) or two days after (length of ninth primary feather) the experimental reduction of haemoglobin using phenylhydrazine hydrocholoride (PHZ). In PHZ broods, all nestlings received injections of PHZ, whereas in control broods, all nestlings received sham injections of saline. In a 2x2 design, nests were either heated every two days to kill all nest-dwelling ectoparasites or not heated to maintain natural parasite loads (control nests).

Response variable	Model variables	Estimate	SE	df	t-value	P	95% CI	
							Lower	Upper
day 6 mass	PHZ+heat treatment	.	.	22.9	.	0.76	.	.
	relative nestling size	1.71	0.09	119.0	18.24	<0.0001	1.52	1.89
day 6 length of head-bill	PHZ+heat treatment	.	.	22.9	.	0.39	.	.
	relative nestling size	0.78	0.05	119.0	16.15	<0.0001	0.68	0.88
day 6 [haemoglobin]	PHZ+heat treatment	.	.	7.9	.	0.19	.	.
	relative nestling size	5.82	2.22	27.1	2.62	0.01	1.27	10.36
day 8 length of ninth primary feather	PHZ+heat treatment	.	.	22.7	.	0.68	.	.
	relative nestling size	1.53	0.09	113.0	16.78	<0.0001	1.35	1.71

Table 3.2 Results of general mixed effects models testing the effects of a 2x2 experiment on the haemoglobin and β-hydroxybutyrate concentrations of nestling tree swallows (*Tachycineta bicolor*). The experiment combined the injection of entire broods with either phenylhydrazine hydrochloride (PHZ broods) or saline as a control (ctl broods) with either a heat treatment that killed all nest-dwelling ectoparasites (heat nests) or a control nest treatment that allowed the natural parasite load to develop (ctl nests). See Methods for calculation of daily weather index.

Response variable	Model variables	Estimate	SE	df	t-value	P	95% CI	
							Lower	Upper
day 6 to 8 [haemoglobin]	PHZ+heat treatment:	.	.	62.7	.	0.03	.	.
	ctl-PHZ vs. ctl-ctl	53.00	24.88	62.3	2.13	0.04	3.27	102.73
	heat-ctl vs. ctl-ctl	7.81	23.32	62.5	0.33	0.74	-38.80	54.41
	heat-PHZ vs. ctl-ctl	51.29	24.02	62.7	2.14	0.04	3.29	99.29
	heat-ctl vs. ctl-PHZ	-45.19	20.49	62.5	-2.21	0.03	-86.15	-4.23
	heat-PHZ vs. ctl-PHZ	-1.71	21.28	62.9	-0.08	0.94	-44.24	40.82
	heat-PHZ vs. heat-ctl	43.48	19.43	63.1	2.24	0.03	4.64	82.32
	age	7.50	1.79	61.3	4.19	<0.0001	3.92	11.08
	treatment*age	.	.	61.3	.	0.01	.	.
day 8 [haemoglobin]	PHZ+heat treatment:	.	.	34.0	.	<0.0001	.	.
	ctl-PHZ vs. ctl-ctl	6.59	4.85	34.0	1.36	0.18	-3.26	16.44
	heat-ctl vs. ctl-ctl	24.46	4.56	34.0	5.36	<0.0001	15.19	33.73
	heat-PHZ vs. ctl-ctl	7.38	4.98	34.0	1.48	0.15	-2.73	17.49
	heat-ctl vs. ctl-PHZ	17.87	3.98	34.0	4.49	<0.0001	9.78	25.97
	heat-PHZ vs. ctl-PHZ	0.79	4.36	34.0	0.18	0.86	-8.07	9.65
	heat-PHZ vs. heat-ctl	-17.08	3.95	34.0	-4.33	<0.001	-25.11	-9.06
	brood size	5.14	2.03	34.0	2.53	0.02	1.01	9.27
day 12 [haemoglobin]	PHZ+heat treatment:	.	.	17.8	.	0.02	.	.
	ctl-PHZ vs. ctl-ctl	-2.05	6.25	17.7	-0.33	0.75	-15.20	11.10
	heat-ctl vs. ctl-ctl	17.51	6.68	17.6	2.62	0.02	3.46	31.55
	heat-PHZ vs. ctl-ctl	16.51	6.56	18.0	2.52	0.02	2.73	30.30
	heat-ctl vs. ctl-PHZ	19.55	6.92	17.5	2.83	0.01	4.98	34.13

	heat-PHZ vs. ctl-PHZ	18.56	6.75	18.2	2.75	0.01	4.39	32.74
	heat-PHZ vs. heat-ctl	-0.99	6.55	18.1	-0.15	0.88	-14.74	12.76
	daily weather index (n-7)	-1.67	0.85	17.6	-1.97	0.06	-3.46	0.11
	brood size	6.87	3.07	17.5	2.24	0.04	0.41	13.33
	relative brood asynchrony	-28.17	12.72	17.6	-2.21	0.04	-54.94	-1.40
	relative nestling size	5.38	1.55	56.3	3.46	<0.01	2.27	8.49
	hatching date	1.94	0.63	17.5	3.05	<0.01	0.60	3.27
day 12 [β-hydroxybutyrate]	PHZ+heat treatment		.	23.8	.	0.24	.	.

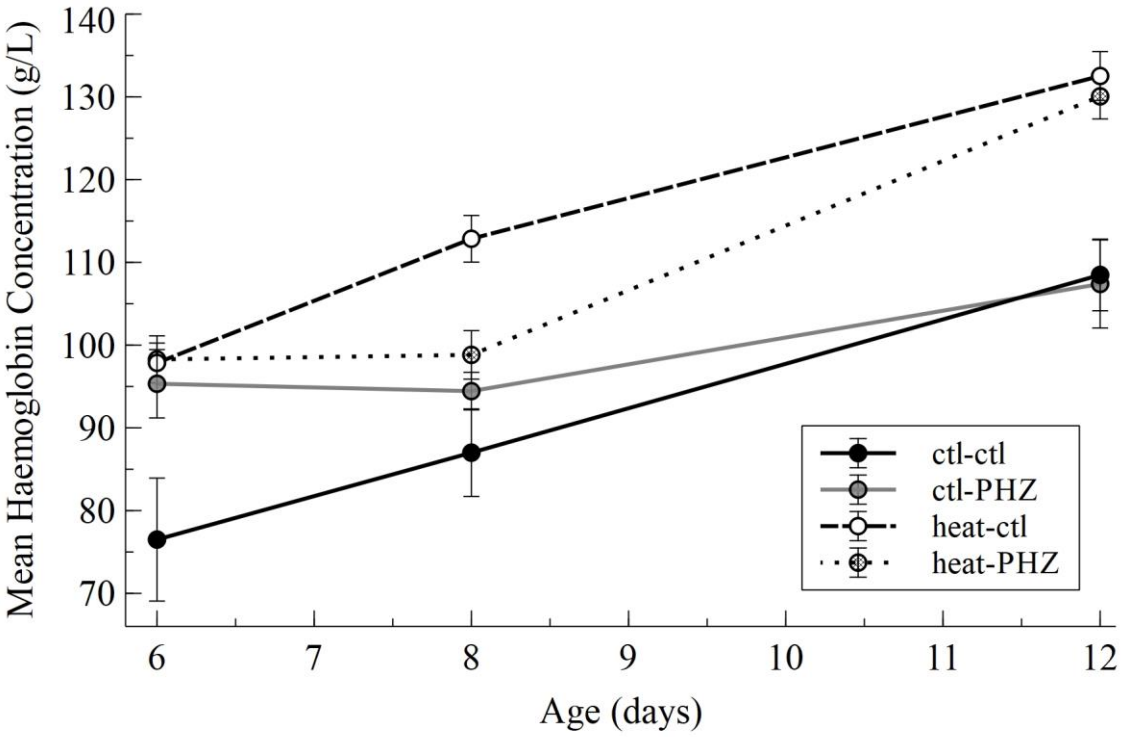

Figure 3.1 The mean haemoglobin concentration (± SE) of nestling tree swallows (*Tachycineta bicolor*) in a 2x2 experiment that combined the injection of entire broods with either phenylhydrazine hydrochloride (PHZ broods) or saline as a control (ctl broods) with either a heat treatment that killed nest-dwelling ectoparasites (heat nests) or a control nest treatment that allowed the natural parasite load to develop (ctl nests). Injection of PHZ prevented the normal increase in haemoglobin concentration with age from day of injection (day 6) to two days post-injection (day 8; n = 6 (ctl-ctl), 9 (ctl-PHZ), 13 (heat-ctl), 11 (heat-PHZ) nestlings). By day 12, however, nestlings had recovered from the PHZ injections and haemoglobin concentration was more influenced by the nest heating treatment, with nestlings from heated nests having significantly higher haemoglobin concentrations than nestlings from control nests (n = 21 (ctl-ctl), 18 (ctl-PHZ), 21 (heat-ctl), 17 (heat-PHZ) nestlings).

12 was positively affected by relative nestling size, hatching date and brood size, and negatively affected by relative brood asynchrony and the daily weather index three days prior to measurement, although this last variable only approached significance (P = 0.07; Table 3.2). β-hydroxybutyrate concentration on day 12 was not influenced by either treatment (P = 0.24).

On day eight, the treatments had no effect on primary ($F_{3,18.0}$ = 1.65, P = 0.21) or secondary ($F_{3,17.6}$ = 0.58, P = 0.63) begging intensity of nestlings (n = 121 nestlings, 27 broods). Instead, primary begging intensity increased marginally with later hatching date (estimate = 0.49 ± 0.26, $F_{1,17.6}$ = 3.65, P = 0.07) and secondary begging intensity decreased marginally with larger brood sizes (estimate = -0.86 ± 0.45, $F_{1,17.9}$ = 3.70, P = 0.07).

The combined treatments had no effect on mass or length of head-bill at day 16 (n = 134 nestlings, 26 broods; Table 3.3). Rather, the daily weather index two days prior to the measurement was positively related to both measurements, while initial relative nestling size (i.e., at day 2) positively predicted length of head-bill. The combined treatment had marginal effects (P = 0.08) on length of ninth primary feather. Control-injected nestlings from parasitized nests had marginally shorter ninth primary feathers than PHZ-injected nestlings from both parasitized (P = 0.08) and heated nests (P = 0.09) and significantly shorter than control-injected nestlings from heated nests (Fig. 3.2). Hatching date and initial relative nestling size were also positively related to length of ninth primary feather (Table 3.3).

Treatments had no effect on growth rate of mass or head-bill (Table 3.4). Growth rate of mass was positively related to hatching date, initial relative nestling size and female body condition, and negatively related to the mean daily weather index from day 4 to day 16, while growth rate of head-bill was positively related to relative nestling size (Table 3.4). Treatment had a significant effect on the growth rate of ninth primary feathers, with control-injected nestlings

Table 3.3 Results of general mixed effects models testing the effects of a 2x2 experiment on the morphological traits of nestling tree swallows (*Tachycineta bicolor*) at 16 days of age. The experiment combined the injection of entire broods with either phenylhydrazine hydrochloride (PHZ broods) or saline as a control (ctl broods) with either a heat treatment that killed all nest-dwelling ectoparasites (heat nests) or a control nest treatment that allowed the natural parasite load to develop (ctl nests). See Methods for calculation of daily weather index.

Response variable	Model variables	Estimate	SE	df	t-value	P	95% CI	
							Lower	Upper
day 16 mass	PHZ+heat treatment	.	.	19.6	.	0.56	.	.
	daily weather index (n-2)	0.28	0.09	19.6	3.11	<0.01	0.09	0.47
day 16 length of ninth primary feather	PHZ+heat treatment:	.	.	20.8	.	0.08	.	.
	ctl-PHZ vs. ctl-ctl	4.29	2.36	20.9	1.82	0.08	-0.61	9.19
	heat-ctl vs. ctl-ctl	6.08	2.27	21.0	2.68	0.01	1.36	10.79
	heat-PHZ vs. ctl-ctl	4.21	2.36	20.8	1.78	0.09	-0.70	9.12
	heat-ctl vs. ctl-PHZ	1.78	2.37	20.8	0.75	0.46	-3.14	6.71
	heat-PHZ vs. ctl-PHZ	-0.08	2.46	20.6	-0.03	0.97	-5.21	5.04
	heat-PHZ vs. heat-ctl	-1.87	2.35	20.6	-0.80	0.43	-6.75	3.01
	hatching date	1.03	0.23	20.8	4.41	<0.001	0.54	1.52
	relative nestling size	3.42	0.25	107.0	13.77	<0.0001	2.93	3.92
day 16 length of head-bill	PHZ+heat treatment	.	.	20.5	.	0.26	.	.
	relative nestling size	0.22	0.05	108.0	4.43	<0.0001	0.12	0.32
	daily weather index (n-2)	0.12	0.05	20.5	2.36	0.03	0.01	0.22

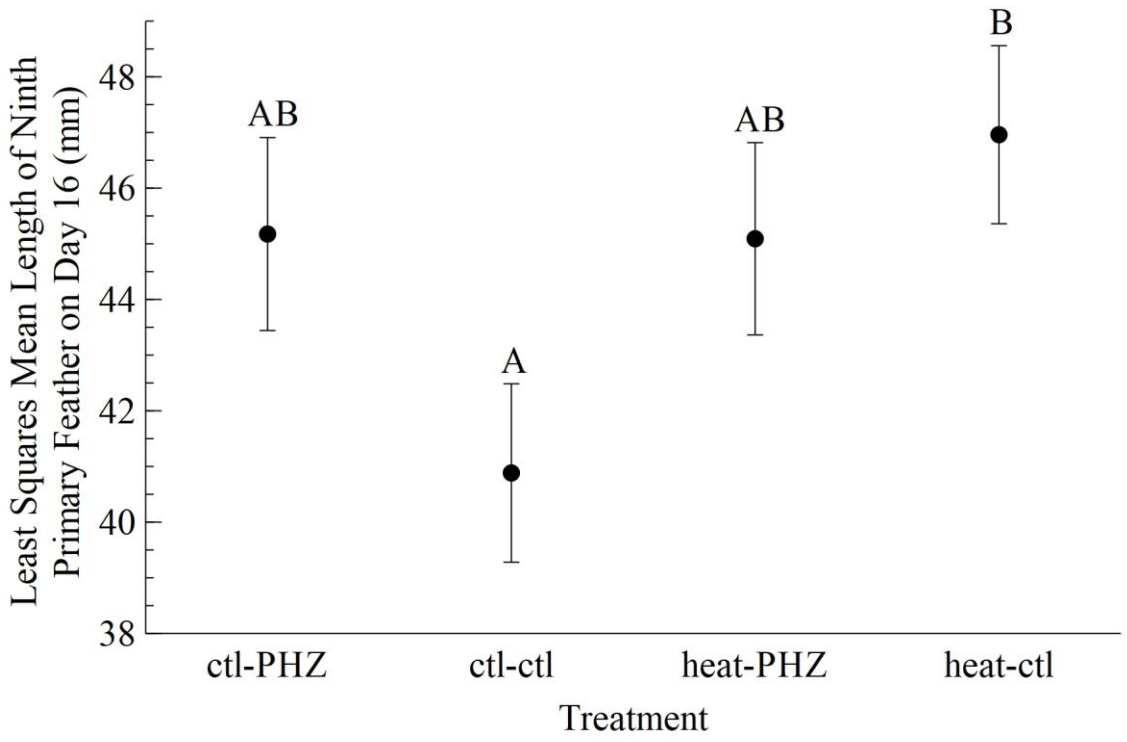

Figure 3.2 The least squares mean length of ninth primary feather (± SE) on day 16 of nestling tree swallows (*Tachycineta bicolor*) during a 2x2 experiment that combined the injection of entire broods with either phenylhydrazine hydrochloride (PHZ broods) or saline as a control (ctl broods) with either a heat treatment that killed nest-dwelling ectoparasites (heat nests) or a control nest treatment that allowed the natural parasite load to develop (ctl nests). Control-injected nestlings from control nests had significantly shorter ninth primary feathers than control-injected nestlings from heated nests and marginally shorter primary feathers than PHZ-injected nestlings, regardless if they were from control or heated nests (n = 33 (ctl-ctl), 31 (ctl-PHZ), 36 (heat-ctl), 34 (heat-PHZ) nestlings). Treatments that share the same letter were not significantly different ($P > 0.05$).

Table 3.4 Results of general mixed effects models testing the effects of a 2x2 experiment on the growth rates of nestling tree swallows (*Tachycineta bicolor*). The experiment combined the injection of entire broods with either phenylhydrazine hydrochloride (PHZ broods) or saline as a control (ctl broods) with either a heat treatment that killed all nest-dwelling ectoparasites (heat nests) or a control nest treatment that allowed the natural parasite load to develop (ctl nests). See Methods for calculation of daily weather index.

Response variable	Model variables	Estimate	SE	df	t-value	P	95% CI	
							Lower	Upper
growth rate of mass	PHZ+heat treatment	.	.	15.9	.	0.66	.	.
	hatching date	0.04	0.01	16.1	2.89	0.01	0.01	0.07
	relative nestling size	0.03	0.01	93.0	3.70	<0.001	0.01	0.04
	mean daily weather index (n-12)	-0.15	0.06	15.9	-2.51	0.02	-0.27	-0.02
	female condition	0.04	0.02	15.9	2.60	0.02	0.01	0.07
growth rate of ninth primary feather	PHZ+heat treatment:	.	.	12.6	.	<0.01	.	.
	ctl-PHZ vs. ctl-ctl	29.48	7.17	13.2	4.11	<0.01	14.01	44.95
	heat-ctl vs. ctl-ctl	30.50	10.87	12.8	2.80	0.02	6.96	54.03
	heat-PHZ vs. ctl-ctl	4.69	16.04	12.2	0.29	0.78	-30.18	39.55
	heat-ctl vs. ctl-PHZ	1.02	10.35	12.6	0.10	0.92	-21.42	23.45
	heat-PHZ vs. ctl-PHZ	-24.79	15.41	12.1	-1.61	0.13	-58.35	8.77
	heat-PHZ vs. heat-ctl	-25.81	17.73	12.2	-1.46	0.17	-64.35	12.73
	hatching date	0.32	0.06	13.6	5.25	<0.001	0.19	0.45
	relative brood asynchrony	1.90	0.70	12.2	2.73	0.02	0.39	3.42
	relative nestling size	0.23	0.03	107.0	8.64	<0.0001	0.18	0.28
	mean daily weather index (n-12)	-0.47	0.22	13.0	-2.14	0.05	-0.95	0.00
	relative brood asynchrony*treatment	.	.	12.6	.	0.06	.	.
	hatching date*treatment	.	.	12.6	.	<0.01	.	.
growth rate of head-bill	PHZ+heat treatment	.	.	21.2	.	0.63	.	.
	relative nestling size	0.01	0.00	108.0	2.37	0.02	0.00	0.01
	mean daily weather index (n-12)	0.01	0.01	21.0	1.47	0.16	0.00	0.03

from parasitized nests having significantly slower feather growth than PHZ-injected nestlings from parasitized nests and control-injected nestlings from heated nests (Table 3.4). Hatching date, relative brood asynchrony, and relative nestling size were positively related to the growth rate of primary feathers, while the mean daily weather index was marginally negatively related ($P = 0.052$). A marginal interaction between treatment and relative brood asynchrony ($P = 0.06$) and a significant interaction between treatment and hatching date retained in the final model ($P = 0.01$), however, suggested that the effect of treatment on growth rate of primaries depended on these two other variables. When analyzed separately by treatment, growth rate of primary feathers was negatively related to relative hatching asynchrony when nestlings from heated nests received control injections (estimate = -1.52 ± 0.51, $F_{1,4.66} = 8.79$, $P = 0.034$), but unrelated in all other treatments (heat-PHZ: estimate = 1.29 ± 2.36, $F_{1,4.05} = 0.30$, $P = 0.614$; control-PHZ: estimate = -0.34 ± 0.69, $F_{1,4.19} = 0.24$, $P = 0.648$; control-control: estimate = 1.66 ± 1.97, $F_{1,4.85} = 0.71$, $P = 0.439$). In contrast, feather growth was positively related to hatching date when nestlings were from parasitized nests (marginally for PHZ-injected nestlings: estimate = 0.06 ± 0.02, $F_{1,4.13} = 6.74$, $P = 0.058$; significantly for control-injected nestlings: estimate = 0.19 ± 0.07 , $F_{1,5.02} = 8.14$, $P = 0.04$) and unrelated in heated nests (heat-PHZ: 0.19 ± 0.09 , $F_{1,3.79} = 4.29$, $P = 0.11$; heat-control: 0.06 ± 0.04 , $F_{1,4.96} = 2.85$, $P = 0.15$; Fig. 3.3).

In terms of adult behaviour, the treatments had no effect on the mean rate of per capita feeding ($F_{3,16} = 0.03$, $P = 0.99$), the mean rate of tremble-thrusting ($F_{3,19} = 0.18$, $P = 0.91$), or the mean rate of sanitation duration ($F_{3,18} = 0.49$, $P = 0.73$; n = 23 broods). Instead, feeding rate was negatively related to hatching date (estimate = -0.18 ± 0.06, $F_{1,16} = 9.68$, $P < 0.01$) and relative brood asynchrony (but only marginally with the latter; estimate = -1.87 ± 1.02, $F_{1,16} = 3.34$, $P =$

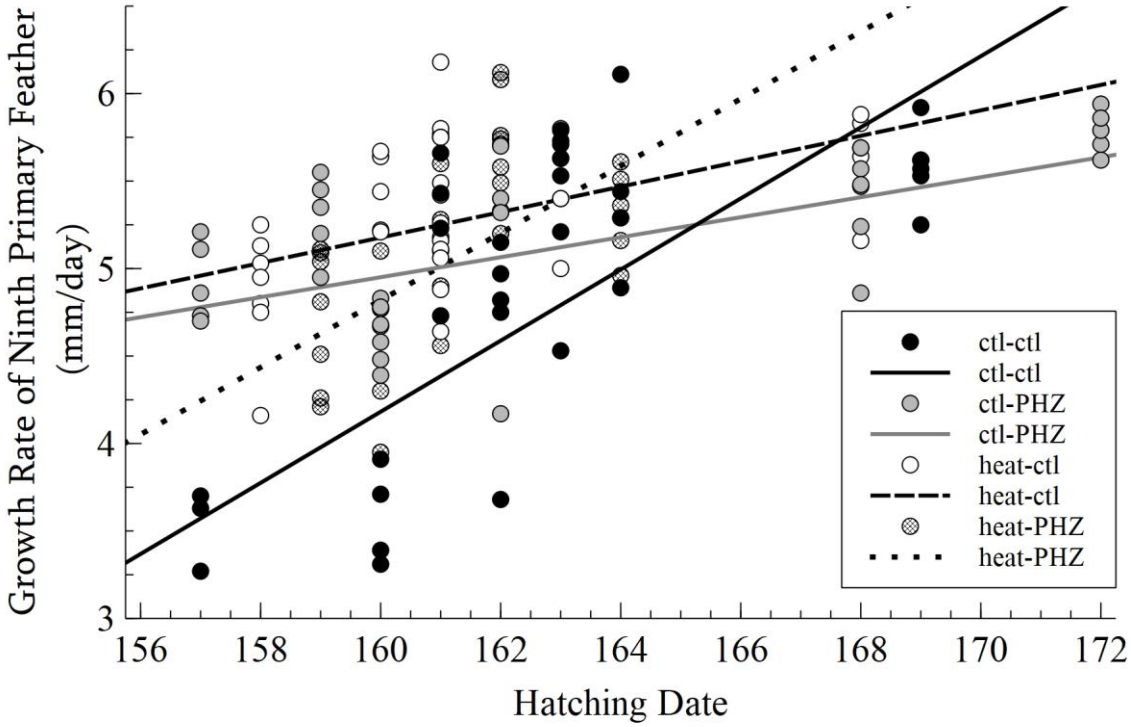

Figure 3.3 The effect of a 2x2 experiment, which combined a heat treatment that reduced ectoparasite load of nests with a phenylhydrazine hydrochloride (PHZ) treatment that reduced haemoglobin concentration, on the growth rate of ninth primary feathers of nestling tree swallows (*Tachycineta bicolor*) depended on hatching date (where 1 = 1 January). Analysis by treatment level showed that growth rate of primary feathers was positively related to hatch date when nestlings were from unheated, control nests (ctl-PHZ and ctl-ctl), but unrelated in heated nests (heat-PHZ and heat-ctl).

0.09), and there was a marginal positive relationship with the mean weather index (estimate = 0.11 ± 0.06, $F_{1,16} = 3.45$, P = 0.08). Rate of tremble-thrusting was unrelated to all covariates included in the model, while rate of sanitation duration was negatively related to brood size (estimate = -57.33 ± 25.78, $F_{1,18} = 4.95$, P = 0.04).

Combined treatments had a marginal effect on the proportion of young fledged from a brood (P = 0.07, n = 23 broods; Table 3.5). Broods with PHZ-injected nestlings in heated nests had significantly higher fledging success than broods with control-injected nestlings in parasitized nests, while fledging success did not differ between broods with control-injected nestlings in heated nests and broods with PHZ-injected nestlings in parasitized nests. All other comparisons had marginal differences in fledging success. The proportion fledged was also positively related to female condition and relative brood asynchrony. A significant interaction term in the final model, however, suggested that the effect of treatment on proportion fledged might depend on hatching date. When analyzed separately by treatment, proportion fledged was positively related to hatching date in broods where nestlings received control injections in parasitized nests (estimate = 0.52 ± 0.16, $F_{1,5} = 10.51$, P = 0.02), but unrelated in all other treatments (heat-PHZ: estimate = -0.27 ± 0.69, $F_{1,4} = 0.15$, P = 0.72; heat-control: estimate = 0.11 ± 0.23, $F_{1,5} = 0.22$, P = 0.66; control-PHZ: estimate = 0.21 ± 0.18, $F_{1,4} = 1.38$, P = 0.31; Fig. 3.4a). The effect of the heat treatment alone on proportion fledged was examined to allow a larger sample size per treatment level, and showed that broods from heated nests fledged a higher proportion of offspring than parasitized nests (Table 3.5). Hatching date, female condition and length of ninth primary feather were all positively related to the proportion of young fledged, while male condition was negatively related (Table 3.5). The final model also retained a significant interaction term between heat treatment and hatching date. When analyzed separately

Table 3.5 Results of general mixed effects models testing the effects of a 2x2 experiment on the proportion of young fledged from broods of tree swallows (*Tachycineta bicolor*). The experiment combined the injection of entire broods with either phenylhydrazine hydrochloride (PHZ broods) or saline as a control (ctl broods) with either a heat treatment that killed all nest-dwelling ectoparasites (heat nests) or a control nest treatment that allowed the natural parasite load to develop (ctl nests).

Response variable	Model variables	Estimate	SE	df	t-value	P	95% CI	
							Lower	Upper
proportion fledged	PHZ+heat treatment:	.	.	13.0	.	0.07	.	.
	ctl-PHZ vs. ctl-ctl	149.67	69.51	13.0	2.15	0.05	-0.51	299.84
	heat-ctl vs. ctl-ctl	128.03	68.87	13.0	1.86	0.09	-20.76	276.81
	heat-PHZ vs. ctl-ctl	425.85	157.12	13.0	2.71	0.02	86.42	765.28
	heat-ctl vs. ctl-PHZ	-21.64	85.05	13.0	-0.25	0.80	-205.38	162.10
	heat-PHZ vs. ctl-PHZ	276.18	130.92	13.0	2.11	0.05	-6.65	559.01
	heat-PHZ vs. heat-ctl	297.82	151.85	13.0	1.96	0.07	-30.23	625.87
	hatching date	0.66	0.41	13.0	1.61	0.13	-0.23	1.54
	female condition	2.22	0.69	13.0	3.23	<0.01	0.73	3.70
	relative brood asynchrony	5.14	2.63	13.0	1.95	0.07	-0.54	10.83
	hatching date*treatment	.	.	13.0	.	0.07	.	.
proportion fledged	heat: heat vs. ctl	324.98	76.29	15.0	4.26	<0.001	162.36	487.60
	hatch date	1.33	0.23	15.0	5.72	<0.0001	0.84	1.83
	female condition	3.36	0.63	15.0	5.29	<0.0001	2.01	4.71
	female ninth	0.27	0.09	15.0	2.86	0.01	0.07	0.46
	male condition	-1.24	0.30	15.0	-4.17	<0.001	-1.87	-0.60
	hatching date*treatment	.	.	15.0	.	<0.001	.	.

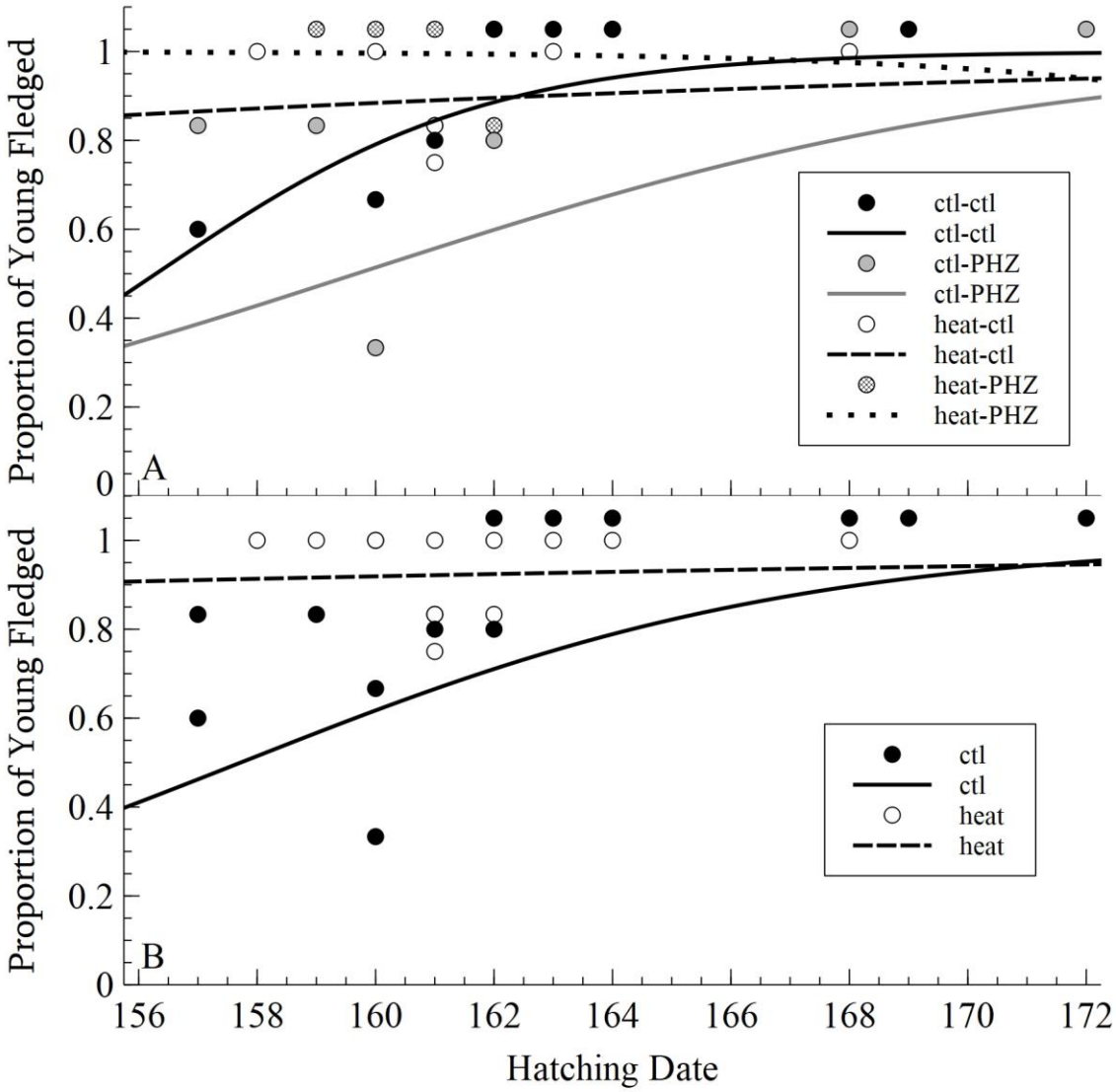

Figure 3.4 The effect of a 2x2 experiment, which combined a heat treatment that reduced ectoparasite load of nests with a phenylhydrazine hydrochloride (PHZ) treatment that reduced haemoglobin concentration, on the proportion of young fledged in the broods of tree swallows (*Tachycineta bicolor*) depended on hatching date (where 1 = 1 January) (A). Analysis by treatment level showed that proportion fledged was positively related to hatch date in broods where nestlings in unheated, control nests received control injections (ctl-ctl), but unrelated in all other treatments (ctl-PHZ, heat-PHZ and heat-ctl). A similar result was produced when only the heat treatment was included in the analysis (B), where proportion fledged was positively related to hatch date in control nests (ctl) and unrelated in heated nests (heat). Data points have been offset slightly from 1 to show their distribution.

by treatment, proportion fledged was positively related to hatching date in parasitized nests (estimate = 0.33 ± 0.14, $F_{1,11} = 5.95$, P = 0.03) and unrelated in heated nests (estimate = 0.06 ± 0.22, $F_{1,11} = 0.08$, P = 0.78; Fig. 3.4b).

In heated nests, no larval blow flies were present, while the mean number per nestling in control nests was 6.02 (± 0.83 SE), a difference that was significant (Mann-Whitney U = 260.0, P < 0.0001, n = 26 broods; Fig. 3.5).

3.5 Discussion

Two treatments, one reducing the parasite load of nests (Fig. 3.5) and the other reducing the haemoglobin concentration of nestlings (Fig. 3.1), a common physiological correlate of haematophagous ectoparasites, had various effects depending on the nestling trait examined. Both treatments affected nestling haemoglobin concentration, and growth and length of flight feathers, while only the parasite reduction treatment affected fledging success. Neither treatment affected begging behaviour of nestlings, or the feeding and sanitation behaviours of adults.

Injection with phenylhydrazine hydrochloride (PHZ) successfully prevented the normal increase in haemoglobin concentration of nestling tree swallows two days post-injection, but by six days post-injection, nestlings appeared to have recovered from the chemical's effects (Table 3.2; Fig. 3.1). To my knowledge, PHZ has only been used previously in adult wild birds (Williams et al. 2012), not nestlings. In European starlings, adults injected with PHZ had a sharp decrease in haemoglobin concentration one day post-injection (~3.5 g/dL change) and then a steady increase until 10 days post-injection when haemoglobin levels were similar to control birds (Williams et al. 2012). Nestlings provide a dynamic situation compared to adults because haemoglobin concentration increases with age naturally during the nestling stage (e.g., Simmons

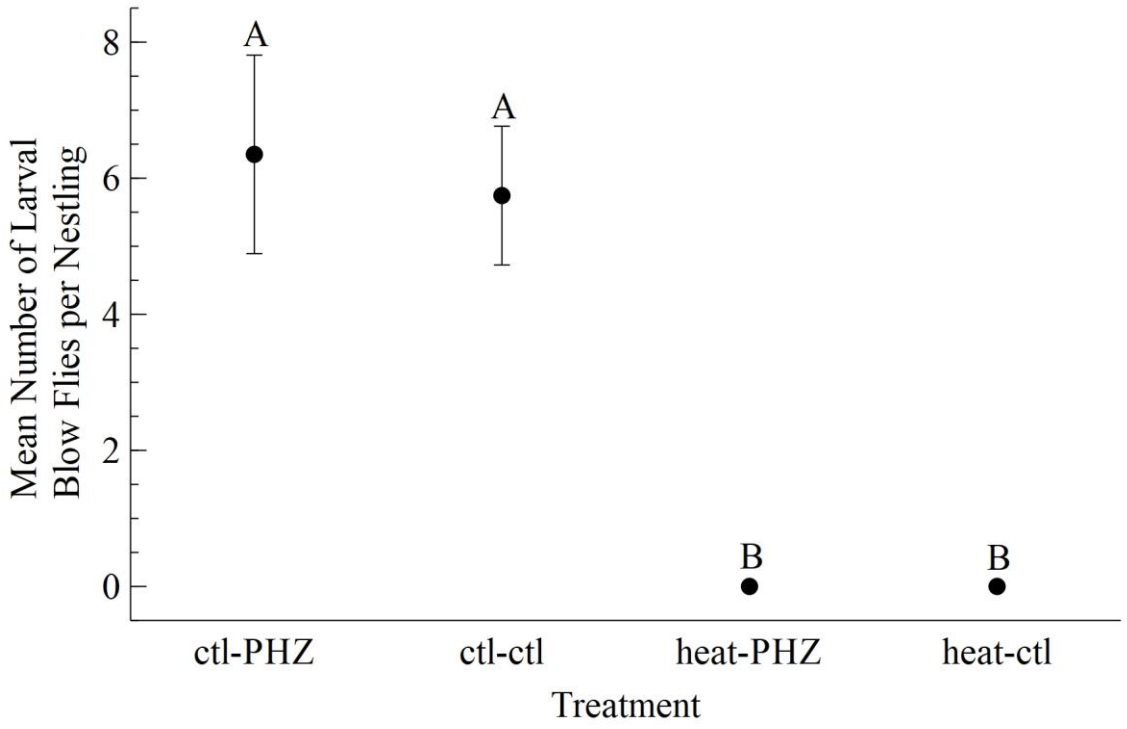

Figure 3.5 The least squares mean number of larval blow flies (± SE) per nestling tree swallow (*Tachycineta bicolor*) during a 2x2 experiment that combined the injection of entire broods with either phenylhydrazine hydrochloride (PHZ broods) or saline as a control (ctl broods) with either a heat treatment that killed nest-dwelling ectoparasites (heat nests) or a control nest treatment that allowed the natural parasite load to develop (ctl nests). Heated nests had significantly lower parasite loads than control nests, while PHZ treatment had no effect on the number of larval blow flies (n = 7 (ctl-ctl), 6 (ctl-PHZ), 7 (heat-ctl), 6 (heat-PHZ) broods). Treatments that share the same letter were not significantly different ($P > 0.05$).

and Lill 2006) and my results suggest nestlings recover more quickly from the effects of PHZ (6 days) compared to adults (10 days; Williams et al. 2012). Interestingly, parasite manipulation did not significantly prevent the normal increase in haemoglobin concentration from day 6 to day 8 (Fig. 3.1), suggesting that either nest-dwelling, blood-feeding parasites did not consume a sufficient volume of blood to significantly reduce haemoglobin concentration or nestlings had enough resources to expend regenerating the haemoglobin lost to parasites. Examining absolute haemoglobin concentration, rather than the change in haemoglobin concentration, showed that at day 8, but not at day 6, control-injected nestlings in heated nests had significantly higher haemoglobin levels than all other treatment groups. This suggests that parasites may have started to negatively affect nestlings physiologically by day 8, but the effects of the PHZ treatment prevented complete detection of such effects, as the haemoglobin concentration of PHZ-injected nestlings at day 8 did not differ between heated and parasitized nests. By day 12 post-hatch, however, when nestling tree swallows typically reach their asymptotic mass (Zach and Mayoh 1982), parasite reduction had a significant positive effect on nestling haemoglobin concentration (Fig. 3.1). Thus, nestlings appeared to be unable to compensate for the haemoglobin lost to parasites at day 12, perhaps because of the high cost of growth and development at that age compared to at six to eight days post-hatch and/or more mature larval blow flies may be taking larger blood meals. A similar pattern was found in black-billed magpies (*Pica hudsonia*), where haemoglobin concentration of nestlings did not differ between nests infested by blow flies and non-infested nests at younger ages, but did significantly differ at older ages (Whitworth and Bennett 1992).

Neither the PHZ nor the heat treatment affected nestling begging behaviour. Previous studies have found both reduced (Helfenstein et al. 2008; O'Connor et al. 2014), as well as

increased begging in parasitized nests (Christe et al. 1996a; Cantarero et al. 2013). Specifically in tree swallows, begging intensity was not associated with ectoparasite loads (Thomas and Shutler 2001), similar to what I found. Although nestling tree swallows use significantly more oxygen when begging than resting at both 5 and 10 days of age (Leech and Leonard 1996), experimental reduction of haemoglobin concentration did not appear to limit begging. Perhaps nestling tree swallows do not pay a high cost of begging in terms of the aerobic activity required or morphological growth trade-offs, but rather pay via another aspect of development (e.g., oxidative stress; Noguera et al. 2010) or increased risk of predation (Leech and Leonard 1997).

Nestlings that received control injections had significantly shorter ninth primary feathers near to fledging if they were from parasitized nests compared to heated nests, suggesting that the energy lost to parasites reduced the ability of nestlings to invest in feather growth (Table 3.3; Fig. 3.2). Nestlings from parasitized nests that received PHZ injections unexpectedly showed marginally longer primary feathers near to fledging compared to control-control nestlings, displaying greater investment into feather growth even when being parasitized (Fig. 3.2). This pattern of feather development could be due to random selection resulting in the poorest performers accumulating in the control-control group, as indicated by the lower, albeit not significantly lower, haemoglobin concentration of control-control nestlings at day 6 (Fig. 3.1; Table 3.1). Alternatively, feather growth can be a flexible trait influenced by the rearing environment (Kinnard and Westneat 2009), and the reduction in haemoglobin concentration from day six to eight triggered a different strategy by nestlings to trade off another aspect of growth or development for increased investment in feather development. Nestlings that face ideal conditions (e.g., heat-control nestlings) have the most energy to invest in feather growth and therefore, have the longest flight feathers near to fledging. Nestlings that sense high parasite

loads at a young age, perhaps signaled physiologically by a reduction in haemoglobin concentration (e.g., PHZ-injected nestlings), may invest more energy into feather growth, likely at a cost to other components of growth and development. For example, nestling barn swallows (*Hirundo rustica*) grew rectrix feathers faster, but tarsus and mass slower, when facing experimentally elevated levels of hippoboscid flies (Diptera) compared to control nestlings, potentially allowing them to fledge earlier and leave their parasite-infested nest sooner (Saino et al. 1998). Interestingly, control-PHZ nestlings did not show reduced growth in either mass or head-bill, suggesting a morphological growth trade-off did not occur. Nestlings that face parasite loads at moderate levels and do not receive any signal at a young age of unusually high parasite loads (e.g., control-control nestlings) may reduce feather growth as a consequence of being more energy-limited, and fledge later. As I did not record exact day of fledging, I cannot confirm if control-control nestlings took longer to fledge. In alpine swifts (*Apus melba*), however, parasitized broods had longer rearing periods compared to parasite-free broods (Bize et al. 2004). In contrast, barn swallow broods had shorter nestling periods (fledged earlier) when raised with experimentally elevated levels of mites compared to nests that were fumigated to remove mites (Møller 1990).

The growth of flight feathers showed a similar pattern as length of flight feathers near to fledging. Nestlings receiving control-injections from control broods grew their ninth primary feathers significantly slower than PHZ-injected nestlings from parasitized nests and control-injected nestlings from heated nests (Table 3.4). This shows that nestlings that had longer flight feathers near to fledging also grew those feathers faster (with the exception of heat-PHZ nestlings, which had greater length, but not faster growth). The effect of the treatments on the growth of primary feathers, however, depended on relative brood asynchrony and hatching date.

Relative brood asynchrony was unrelated to feather growth in all treatments except heated nests, where growth of control-injected nestlings was slower when nestlings were from more asynchronous broods and faster when they were from more synchronous broods. While the underlying cause of these patterns is not clear, they suggest that brood synchrony is beneficial in parasite-free states. Sibling competition in asynchronous broods may prevent the smaller nestlings within a brood, which are at a competitive disadvantage, from obtaining enough resources from parents to reach higher rates of flight feather growth (e.g., Cotton et al. 1999), resulting in lower quality nestlings overall relative to synchronous broods. Alternatively, nestlings in synchronous broods, where nestlings are all similar in size and competitive ability, may actually compete more intensively due to the lack of a stable dominance hierarchy (the 'sibling rivalry reduction hypothesis'; Hahn 1981). Furthermore, unlike asynchronous broods, the timing of when a nestling reaches peak development and requires the most food is not staggered among nest mates, making it more difficult for parents to supply a sufficient amount of food (the 'peak load hypothesis'; Hussell 1972). A lack of parasites may allow nestlings to grow flight feathers more quickly when competition is intense in an attempt to fledge earlier and escape the high competitive environment within the nest.

In parasite-infested environments (control nests), growth of feathers was positively related to hatching date, whereas it was not in parasite-free nests (heated nests; Fig. 3.3). The effect of the treatments on fledging success also depended on hatching date, with parasites reducing fledging success early in the season, but not later (Table 3.5). A previous study on this population of tree swallows and another in Saskatchewan found that the number of larval blow flies decreased for birds breeding later in the season (Harriman et al. 2014). In parasitized nests, nestlings may therefore face fewer parasites the later they hatch and have more energy available

to invest into feather growth, whereas in heated nests, nestlings experience a more stable environment with regards to parasites, regardless of hatching date, and thus, growth rate of primaries did not vary with hatching date. Over the 2017 breeding season, however, the number of larval blow flies did not decrease significantly with hatching date ($r = -0.13$, $P = 0.67$, $n = 13$ broods). This suggests that some other component besides the parasite environment improved as the season progressed. Two possibilities are that the abundance or quality of food increased later in the season and/or higher quality parents bred later than lower quality parents. Both of these potential explanations, however, contradict the common observation that nestling quality and fledging success declines over the breeding season and is often explained by decreases in environmental conditions, such as food availability (the 'date hypothesis'; Perrins 1970) or parental quality (the 'quality hypothesis'; Price et al. 1988). For my experiment, the latest brood included was hatched on 21 June, whereas tree swallow clutches continued to hatch until 14 July during the 2017 season. The experiment was truncated to ensure that, as the number of broods hatching declined later in the season, the treatment could be applied to nest pairs matched for hatching date (± 1 day) and to avoid late season broods that tend to be of poorer quality. Therefore, the positive relationship I found with hatching date may have been an artifact of my sample not providing a full representation of the entire breeding season.

Contrary to expectation, both feeding rate and nest sanitation by adults were unaffected by both treatments. In some bird species, when facing an infestation of parasites, parents favour self-maintenance and do not change or sometimes even decrease feeding rates (e.g., Møller 1994), while others prioritize the current reproductive attempt, increasing feeding rates (e.g., Bouslama et al. 2002). My results suggest that adults in this population were favouring self-maintenance. In a different tree swallow population, DeSimone et al. (2018) also found no

change in feeding rate when nest infestation levels of blow flies were experimentally manipulated. Previous studies have found that females decrease sanitation behaviour (actively searching the nest material) in response to experimentally reduced parasite loads, particularly at night at a cost to the amount of time spent sleeping (e.g., Christe et al. 1996b). Potentially in my population, adults were constrained by feeding the nestlings during the day, and therefore, could not invest more time in nest sanitation, and instead increased nest sanitation in infested nests only at night when I did not take video recordings. A previous study in the same population, however, found no difference in sanitation behaviour between heated and parasitized nests during the night (Williams 2017). This suggests that adults were not trying to reduce the negative effects of parasites on their young using behavioural changes, and rather invested more in self-maintenance instead of their current reproductive effort (e.g., Møller 1994).

In conclusion, I found that both haemoglobin concentration and parasites affected nestling morphology, specifically growth of flight feathers and their length near fledging. These results may indicate that a sudden reduction in haemoglobin concentration at a young age may act as a physiological cue to nestlings to switch to a different feather growth strategy, although further research is required to explore this possibility fully. Furthermore, the effect of the treatment on the growth rate of primaries varied with both time of season and relative brood asynchrony. The PHZ treatment successfully reduced nestling haemoglobin concentration two days post-injection, but no longer had an effect by six days post-injection, at which time only the parasite manipulation affected haemoglobin concentration. To my knowledge, this is the first time PHZ has been used in nestling wild birds and it provides an excellent tool for experimentally investigating the effects of reduced haemoglobin concentration. Because nestlings recover more quickly than adults from the effects of the chemical, a second injection

may be necessary to better replicate the effects of haematophagous parasites. I hope the validation of this technique in nestlings will prompt more experiments with the objective of teasing apart the effects of parasitism and reduced haemoglobin, an understudied, but important, area of research.

4. Predictors of nestling survival during harsh weather events in an aerial insectivore, the tree swallow (*Tachycineta bicolor*)

4.1 Abstract

Extreme weather events can influence the population dynamics of wild animals. For organisms whose food source is affected by environmental conditions, such as aerial insectivorous birds, periods of inclement weather can have devastating effects. Here, I examine predictors of survival of individual nestlings and whole broods in tree swallows (*Tachycineta bicolor*) during an extreme, two-day harsh weather event in central British Columbia, which co-occurred with experimental reduction of nest ectoparasite loads using an anti-parasite drug (ivermectin) or heat-treating nests. A curvilinear relationship existed between survival and brood age, such that middle-aged broods were least likely to survive. Survival of broods and individual nestlings was higher when raised by males with bluer plumage, whereas it was lower when female parents had brighter and more UV-reflective plumage. Within broods, smaller nestlings had a lower chance of surviving than their larger siblings. Nestlings in broods where half of the offspring received ivermectin injections had significantly higher chances of surviving than nestlings from non-experimental broods, suggesting that parasite loads can influence survival during inclement weather. My results identify several factors influencing resiliency of nestlings to harsh weather and are particularly relevant in the context of declining aerial insectivorous bird populations and climate change.

4.2 Introduction

Extreme weather events can play a major role in the population dynamics of wild animals

and are particularly concerning in the context of climate change (e.g., Easterling et al. 2000), as the frequency of such events is predicted to increase (IPCC 2014). Weather conditions can affect the reproductive success of individuals, as demonstrated across a diversity of taxa (insects: Weisser et al. 1997; reptiles: Rock and Cree 2003; mammals: Grassel et al. 2016; amphibians: Greenberg et al. 2017; fish: Moll et al. 2018). Specifically in birds, environmental variables, such as temperature and precipitation, can influence nestling phenotype (McCarty and Winkler 1999; Ambrosini et al. 2006) and reproductive success of adults (Dawson and Bortolotti 2000; Arbeiter et al. 2016). For example, during inclement weather (cool, windy, and rainy conditions), nestling American kestrels (*Falco sparverius*) were less likely to fledge (Dawson and Bortolotti 2000). Young birds may differ, however, in their tolerance of poor weather conditions, due to both intrinsic (e.g., age; Boyle et al. 2013) and extrinsic (e.g., ectoparasites; Merino and Potti 1996) factors, which in turn may influence their survival. Although these factors can affect nestling survival, they are less well studied in the context of extreme or harsh weather events.

Intrinsic factors that can affect brood and nestling survival include their age, brood size and within-brood size hierarchies. Survival of nestlings, or number of young fledged, generally increases with brood size, though often at the expense of individual chick size (Reid 1988; Arnold 1993; Gilliland et al. 2016). Specifically during cold snaps, larger broods may have a better chance of surviving by reducing heat loss through huddling behaviour (Gilbert et al. 2010). Depending on an organism's pattern of morphological and physiological development, nestlings may be more vulnerable to poor weather conditions at certain ages. For example, 6–9 day old tree swallows (*Tachycineta bicolor*), which have developed some thermoregulatory ability (Dunn 1979), but for which growth of feathers is insufficient to provide adequate insulation, had the highest mortality risk during periods of cold weather (Boyle et al. 2013). When size

hierarchies exist within a brood due to asynchronous hatching, brood reduction may occur more efficiently, as the smallest nestling within a brood is expected to die quickly due to its competitive disadvantage and lack of resources (the 'brood reduction hypothesis'; Lack 1947). The rapid death of the smallest nestling in asynchronous broods may allow parents to cope with poor environmental conditions better than parents of more synchronous broods, where brood reduction will occur more slowly (Slagsvold 1982, 1986; Gibbons 1987). In red-winged blackbirds (*Agelaius phoeniceus*), when temperatures were cool, more marginal (i.e., last-hatched) nestlings died, whereas no such effect was observed on core (i.e., first-hatched) nestlings (Forbes et al. 2001).

Extrinsic factors, such as condition and quality of parents, can also affect brood and nestling survival. Parental phenotype can influence reproductive success, either because the trait provides some direct advantage to the adult (e.g., morphologically) or because it provides an indirect advantage via sexual selection (e.g., plumage colouration). For example, larger adult size has been associated with greater reproductive success in many bird species (Forstmeier 2002; Blums and Clark 2004; but see Mänd et al. 2005). Plumage characteristics, such as brightness and badge size, have also been associated with reproductive success and females may use ornaments displayed by males to select higher quality mates that provide better parental care (the 'good parent hypothesis'; Hoelzer 1989). In support, plumage ornamentation of male birds has been positively correlated with male feeding rate (e.g., Siefferman and Hill 2003; Silva et al. 2008) and the number of young fledged (e.g., Silva et al. 2008; Moreno et al. 2013). Females can also display variation in plumage ornamentation. While these visual ornaments may arise due to genetic correlations between the sexes (Lande 1980; Dale et al. 2015), female plumage ornamentation may also signal social dominance if female birds compete for limited resources,

such as food, mates, or nesting cavities (Irwin 1994). Lastly, the 'good parent hypothesis' could also explain female ornamentation, with male birds selecting females with more elaborate plumage ornamentation if it is a signal of the quality of parental care a female can provide (Owens and Thompson 1994; Johnstone et al. 1996; Massaro et al. 2003; reviewed in Tobias et al. 2012). Indeed, female plumage has been positively correlated with female feeding rate, clutch size, number of fledglings produced, and nestling mass and immune response (Linville et al. 1998; Silva et al. 2008; Remeš and Matysioková 2013; Morrison et al. 2014). The relationship between plumage ornamentation and reproductive success may depend on current environmental conditions, as different phenotypes may arise as adaptions for different environmental contexts. For example, nestling pied flycatchers (*Ficedula hypoleuca*) raised by black males were lighter than nestlings raised by brown males when the temperatures were relatively low, but when the temperatures were relatively high, the reverse pattern was observed, with brown males raising lighter nestlings than black males (Järvistö et al. 2015). During a year with poor weather and unfavourable breeding conditions, female plumage colouration of great tits (*Parus major*) was negatively related to number of fledglings, whereas the year prior when conditions were favourable, no relationship between plumage and reproductive success was observed (Mänd et al. 2005).

The nest environment, which includes parasite load and microclimate, is another extrinsic factor that can play a role in nestling and brood survival. Ectoparasites can negatively affect nestling survival (Shields and Crook 1987; Pinkowski 1977) and negative effects of parasites on nestlings can be amplified in years with poor environmental conditions, such as lower temperatures and higher rainfall (Dufva and Allander 1996; Allander 1998; Merino and Potti 1996). The composition of nest material (Mainwaring and Hartley 2008; Heenan et al. 2015)

and, for cavity nesting birds, the physical characteristics of the cavity itself (e.g., wall thickness; Wiebe 2001; Fairhurst et al. 2012) can influence nest microclimate with important consequences for nestling phenotype (e.g., Ardia et al. 2010; Fairhurst et al. 2012), survival (e.g., Siikamaki 1996; Dawson et al. 2005b), and fledging success (e.g., Ardia 2013; Deeming and Pike 2015).

Poor weather conditions can be particularly detrimental for species where the availability of their food source depends on environmental conditions. Aerial insectivorous birds are particularly vulnerable to short-term changes in temperatures, as the availability of flying insects is reduced during cool, windy conditions (Bryant 1975, 1978; Arbeiter et al. 2016). A study on tree swallows in Ithaca, NY, USA, identified 18.5°C as the critical temperature below which flying insect availability begins to decline (Winkler et al. 2013). Nestling survival was best predicted by three consecutive days with maximum daily temperatures below 18.5°C (i.e., "cold snaps") and brood age, whereas brood survival was best predicted by cold snap durations of one or two days, brood age, and year (Winkler et al. 2013). This demonstrates that mortality in nestling aerial insectivores was determined not only by temperature and duration of cold periods, but also by age of young, an intrinsic factor.

Here, I investigate the factors that were most important in influencing nestling and brood survival in tree swallows during a harsh weather event. During 2016, two days of harsh weather resulted in the death of 49% and 86% of the broods at two field sites in central British Columbia, Canada. On June 15th, the temperature range was 4.1–9.1°C, with an average of 6.6°C, and on June 16th, it ranged from 4.3–10.9°C, with an average of 7.6°C (Environment and Climate Change Canada 2017). Total precipitation was 25.7 and 20.3 mm on these two days, respectively (Environment and Climate Change Canada 2017). This harsh weather event provided a unique opportunity to investigate how the characteristics of a nestling's parents, brood and nest

environment influenced survival of both individual nestlings and broods during inclement weather, which can all be important in determining nestling success (e.g., Pinkowski 1977; Forbes et al. 2001; Blums and Clark 2004; Losdat et al. 2013). The relative importance, however, of these influential factors on nestlings during a harsh weather event have to my knowledge not been simultaneously examined. Furthermore, this harsh weather event co-occurred with an experiment reducing loads of ectoparasites at both the level of the individual nestling and the brood, using a broad spectrum, anti-parasite drug, ivermectin and heat treatment of nests, respectively. Understanding how aerial insectivorous birds, whose populations are declining across North America (Nebel et al. 2010), respond to such events may be particularly important in the face of climate change, as the frequency of extreme weather events is predicted to increase (IPCC 2014).

As indicators of adult condition, quality, age, and initial reproductive investment (e.g., Bitton and Dawson 2008; Bitton et al. 2008; Canestrari et al. 2011; Berzins and Dawson 2016), I predicted that parental mass, size (length of ninth primary flight feather), and plumage colour, as well as yolk and egg size, would be positively associated with survival of young. I further predicted that larger brood size would increase the chance of the brood surviving through huddling behaviour (Gilbert et al. 2010), but might decrease survival of last-hatched offspring (the 'brood reduction hypothesis'; Lack 1947), as tree swallows show moderate hatching asynchrony (Zach 1982). I expected middle-aged broods, where nestlings have begun expending energy to thermoregulate but have poor feather insulation to retain the energetically costly heat they generate (Boyle et al. 2013), to have lower survival than nestlings in older and younger broods. Previous studies have demonstrated that the negative effects of parasites, such as larval blow flies (*Protocalliphora* and *Trypocalliphora* spp.; Whitworth 2003), on nestlings can be

amplified in years with poor environmental conditions, such as lower temperatures and higher rainfall (Dufva and Allander 1996; Merino and Potti 1996; Allander 1998). Therefore, I predicted that nestlings either injected with ivermectin, an anti-parasite drug, or from broods where the nest was heated to kill ectoparasites would be less parasitized and have higher survival than control and non-experimental nestlings that must cope with the combined costs of harsh weather and parasites.

4.3 Materials and methods

4.3.1 Study area and species

I studied the tree swallow, an aerial insectivorous bird, near Prince George, BC, Canada (53°N, 122°W) at two study sites within 10 km of each other (colloquially referred to as Stewards and Western) between May and August of 2016. Tree swallows are secondary-cavity nesters that also readily breed in nest boxes. The Stewards site was composed of predominantly hay fields with 139 nest boxes mounted on fence posts. Approximately 75% of these nest boxes were situated in hay fields, while the remaining nest boxes were positioned along a fence line that ran between a rural, gravel road and cattle pasture. The Western site was characterized by a series of managed wetlands, with 60 nest boxes positioned around the perimeters of the wetlands on fence posts. Nest boxes were mounted ~1.5 m above the ground.

Larval blow flies are a common haematophagous ectoparasite of nestlings in my tree swallow population. The life cycle begins with adult females laying eggs in the nest material of birds (Sabrosky et al. 1989). Upon hatching, larvae live in the nest material and feed intermittently on the blood of nestlings, except for one species (*T. braueri*) that imbeds under the

nestling's skin for extended periods of time. After the third instar, larvae pupate and develop into adult flies (Hall et al. 2017).

4.3.2 General field methods

Nest boxes were visited daily beginning in the second week of May to document nest building and egg laying. As eggs were laid, they were individually marked with a non-toxic felt pen. The day after an egg was laid, it was weighed using an electronic balance (nearest 0.01 g) and a digital photograph was taken of the egg within an "ovolux" device (Ardia et al. 2006), which illuminated the egg. Using ImageJ software (Rasband 1997), I took four measurements of the yolk diameter from each photo and used the average of these four measurements to calculate yolk volume.

Once the laying of a clutch was completed, the nest was not checked again until the estimated hatch date (12 days following clutch completion; Winkler et al. 2011), at which point the nest was checked daily to determine actual hatching date. Once all viable eggs had hatched, adult birds were captured in nest boxes when feeding offspring. Adults were banded (or the band number recorded, if previously banded) and measured. Body mass was determined using a spring scale (nearest 0.25 g), length of ninth primary flight feather, tail and outer rectrix were measured using a ruler (nearest 0.5 mm), and the combined length of the head and bill ('head-bill') measured using digital calipers (nearest 0.01 mm). Rump feathers from adults were collected and stored in opaque envelopes at room temperature until used for colour analysis (details below).

At day 2 post-hatch, nestlings were weighed with an electronic balance (nearest 0.01 g). In tree swallows, a negative relationship has been demonstrated between hatching order and nestling mass within a few days of hatching (Bitton et al. 2006), which makes mass at day 2 a

good proxy for the degree of hatching asynchrony within a brood. Nestlings were measured (mass, length of head-bill, and length of ninth primary feather) every two days from day 4 until day 16. Nestlings were weighed using a spring scale (nearest 0.125 g). Length of head-bill was measured using digital calipers (nearest 0.01 mm) and length of ninth primary feather using a ruler (nearest 0.5 mm). Ninth primary measurements did not begin until day 8 when the primaries typically have emerged.

4.3.3 Experimental procedure

At the Stewards field site, broods were matched by hatching date (± 1 day) and brood size (± 1 nestling) to form pairs of treatment and control broods. On day 6, nestlings in treatment broods were ordered by mass and every other nestling was injected with ivermectin (IVM), a broad-spectrum, anti-parasite drug, at a dose of 0.2 mg/kg body mass. This drug was expected to reduce the level of parasitism individual nestlings encountered from larval blow flies (Levot and Sales 2002, 2008). Ivomec® 1% Injection for Cattle and Swine was diluted with sterile sesame oil to a concentration of 0.06 mg ivermectin/1 mL oil and appropriate volumes, calculated for the mass of each nestling, were injected subcutaneously, using a 0.3-mL syringe with a 29-gauge needle. Nestlings in treatment broods that did not receive IVM injections were given pure oil injection to control for possible negative effects of subcutaneous injection and possible positive effects of additional nutrients provided by the oil. In control broods, all nestlings received pure oil injections.

At the Western field site, parasite reduction at the individual level (i.e., IVM treatment) was combined with parasite reduction at the nest level using a heat treatment in a 2x2 design, resulting in four treatment combinations. To reduce nest-dwelling ectoparasites at the nest level,

at half of the nests offspring were temporarily removed and nests were heated in a microwave appliance (powered by a generator) every two days, from day 4 to 16. This is an effective method for reducing the number of nest-dwelling ectoparasites, such as larval blow flies, mites, and fleas (O'Brien and Dawson 2008; Chapter 3), with an average reported effectiveness of 98% (Hund et al. 2015). Nests were placed in a sealable plastic bag to reduce loss of moisture during heating. To ensure equal handling, control nests were removed from nest boxes every two days and returned without heating. Within the two levels of the heat treatment (heated and control nests), the IVM experiment was applied, with broods paired, as described above, and assigned as either control (all nestlings in a brood injected with oil) or treatment broods (half of the nestlings in a brood injected with IVM and half injected with oil).

4.3.4 Plumage spectrometry

The spectral characteristics of rump feathers from adult birds (n = 80 males, 83 females) were measured using an Ocean Optics USB2000 spectrometer (Dunedin, FL) with a deuterium tungsten halogen light source (Avantes, Broomfield, CO). Four feathers from each individual were mounted onto a piece of thick, black paper, in an overlapping position to mimic their natural positioning on the body. A bifurcate probe, which was fixed in a cylindrical sheath to ensure the probe was perpendicular to the feather surface and eliminated ambient light, was used to take three measurements for each set of feathers. Reflectance was determined as the proportion of light reflected relative to a WS-1 white standard (Ocean Optics, Dunedin, FL) over the range of wavelengths perceived by songbirds (300–700 nm; Hart 2001). Using the pavo package (Maia et al. 2013) in R version 3.3.0 (R Core Team 2016), I extracted hue (the wavelength of maximal reflectance; R_{max}), mean brightness (mean relative reflectance over the

range visible to birds; total $R_{300–700\ nm}$ / n_w), blue chroma (the relative contribution of the blue range to overall brightness; total $R_{400–512\ nm}$ / total $R_{300–700\ nm}$), and ultraviolet (UV) chroma (the relative contribution of the UV range to overall brightness; total $R_{300–400\ nm}$ / total $R_{300–700\ nm}$) (Montgomerie 2006). Following the extraction of these variables, the three measurements taken for each bird were averaged for each colour variable.

4.3.5 Statistical analysis

Survival of broods (Stewards = 43 broods, Western = 37 broods) was analyzed using generalized linear mixed effects models (logit link and binomial family) with a binary response variable (0 = all nestlings in a brood died, 1 = at least one nestling in a brood survived) and site included as a random effect (random intercept). As explanatory variables, I initially included IVM brood treatment, nest heat treatment, brood age and age^2, brood size, relative hatching asynchrony (the largest minus the smallest day two mass within a brood, divided by the mean day two brood mass), plumage colouration of each parent (brightness, hue, UV and blue chroma), morphology of each parent (mass, and length of ninth primary feather), mean egg mass, and mean relative yolk volume. Brood age and age^2 were both included to test for nonlinear relationships. Prior to analysis, all explanatory variables were tested for collinearity, but no variables had tolerance values less than 0.2. The interaction between IVM treatment and relative hatching asynchrony was also tested.

Survival of individual nestlings (Stewards = 41 broods, 222 nestlings, Western = 33 broods, 189 nestlings) was analyzed using generalized linear mixed effects models (logit link and binomial family), which allowed for a binary response variable (0 = died, 1 = survived) and random effects for nest identity and site to account for variation due to individual nests and

clustered data effects. As explanatory variables, I included IVM treatment, nest heat treatment, brood age and age^2, brood size, relative hatching asynchrony, nestling relative mass within the brood at day two (each nestling's mass minus mean brood mass, divided by the standard deviation), plumage colouration of both parents (brightness, hue, UV and blue chroma), morphology of both parents (mass and length of ninth primary feather), total egg mass, and mean relative yolk volume. Nestling morphology variables could not be included due to collinearity issues (tolerance < 0.2) with brood age and age^2, but all other variables had tolerance values greater than 0.2. The following interactions were also tested: treatment*relative hatching asynchrony and treatment*relative nestling size. If nestlings were not weighed until day four, then mass at day four, rather than at day two, was used to calculate relative size within the brood and relative hatching asynchrony (n = 33 of 460 nestlings).

For survival of both broods and nestlings, I began with a full model with all variables and interactions of interest included and then used a backward-stepwise procedure to eliminate any interactions and variables that did not approach significance (P > 0.10; Dupont 2009; Montgomery et al. 2012). Using a threshold value of P = 0.10 reduces the risk of excluding important effects and the use of a higher threshold has been recommended to allow the inclusion of variables that approach significance (Golberg and Cho 2004; Moreira et al. 2005). One at a time, I first removed interactions, followed by single variables, in order of decreasing P-value. All analyses were performed using Stata (StataCorp 2015). Sample sizes differ slightly between brood and nestling analyses due to missing data.

4.4 Results

4.4.1 Brood survival

The relationship between brood survival and age at the time of the harsh weather event was curvilinear, such that broods of younger and older ages had a higher chance of at least one offspring surviving the event compared to middle-aged broods (Table 4.1; Fig. 4.1a). The chance of broods surviving also was negatively related to male hue, where broods being raised by bluer males had higher survival (Fig. 4.2a). Neither the IVM nor heat treatment had an effect on brood survival. No female plumage characteristics or any other male plumage characteristics predicted survival. Parent morphology and initial female reproductive investment (i.e., mean egg mass and relative yolk volume), as well as brood size and relative hatching asynchrony, were also not significant in predicting brood survival.

4.4.2 Nestling survival

A curvilinear relationship existed between individual nestling survival and brood age, such that nestlings from relatively young or relatively old broods had a higher chance of survival than nestlings from middle-aged broods (Table 4.2; Fig. 4.1b). The nestling's relative size within the brood positively predicted survival (Fig. 4.3). Nestling survival was also negatively related to hue of the male's plumage, where nestlings being raised by bluer males had higher survival (Fig. 4.2b), while nestlings raised by females that had brighter and more UV-reflective plumage had lower survival (Fig. 4.4). Nestlings from broods where half of the nestlings received IVM-injections had a higher chance of survival, regardless of whether they received a control- or IVM-injection, compared to nestlings from non-experimental broods (Fig. 4.5). The survival of nestlings from control broods (all nestlings received control-injections), however, did not differ

Table 4.1 Results of a generalized linear mixed effects model examining the survival of broods of tree swallows (*Tachycineta bicolor*) during a 2-day harsh weather event in central British Columbia, Canada, during June 2016.

Response variable	n	Model variables	Estimate	SE	z	P	95% CI	
							Upper	Lower
Brood survival	80	age	-1.119	0.365	-3.06	0.002	-1.84	-0.40
		age^2	0.065	0.021	3.12	0.002	0.02	0.11
		male hue	-0.057	0.025	-2.28	0.023	-0.11	-0.01

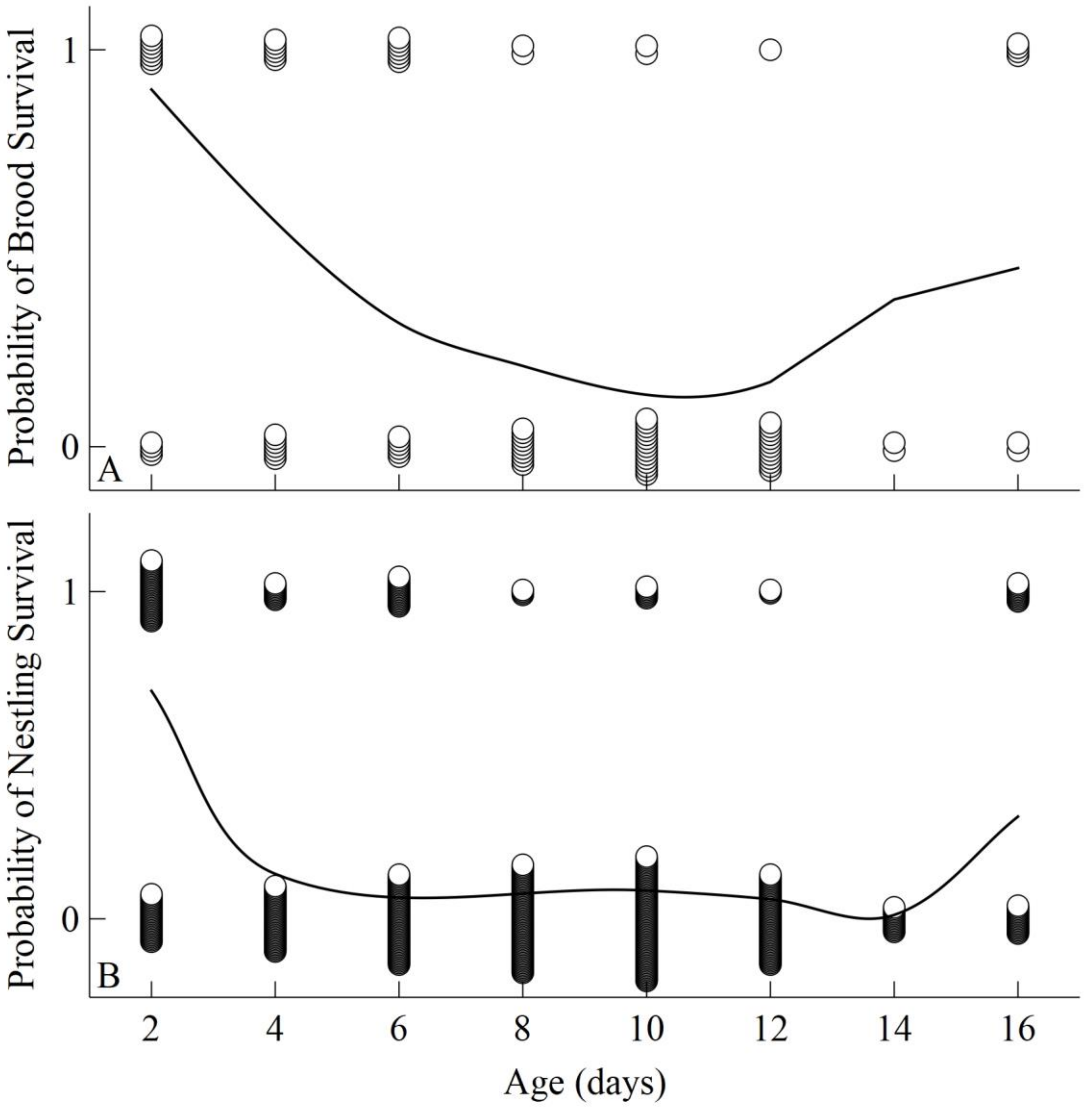

Figure 4.1 The probability that (A) broods and (B) individual nestling tree swallows (*Tachycineta bicolor*) survived a 2-day harsh weather event in June 2016 was higher for younger and older aged broods, but lower for middle-aged broods. Brood survival was defined as all nestlings dying within a brood (0) or at least one nestling surviving in a brood (1). Nestling survival was defined as 0 when a nestling died and 1 when a nestling survived. The prediction lines were estimated using generalized linear mixed effects models (logit link and binomial family). Data points have been offset slightly from 0 and 1 to show their distribution.

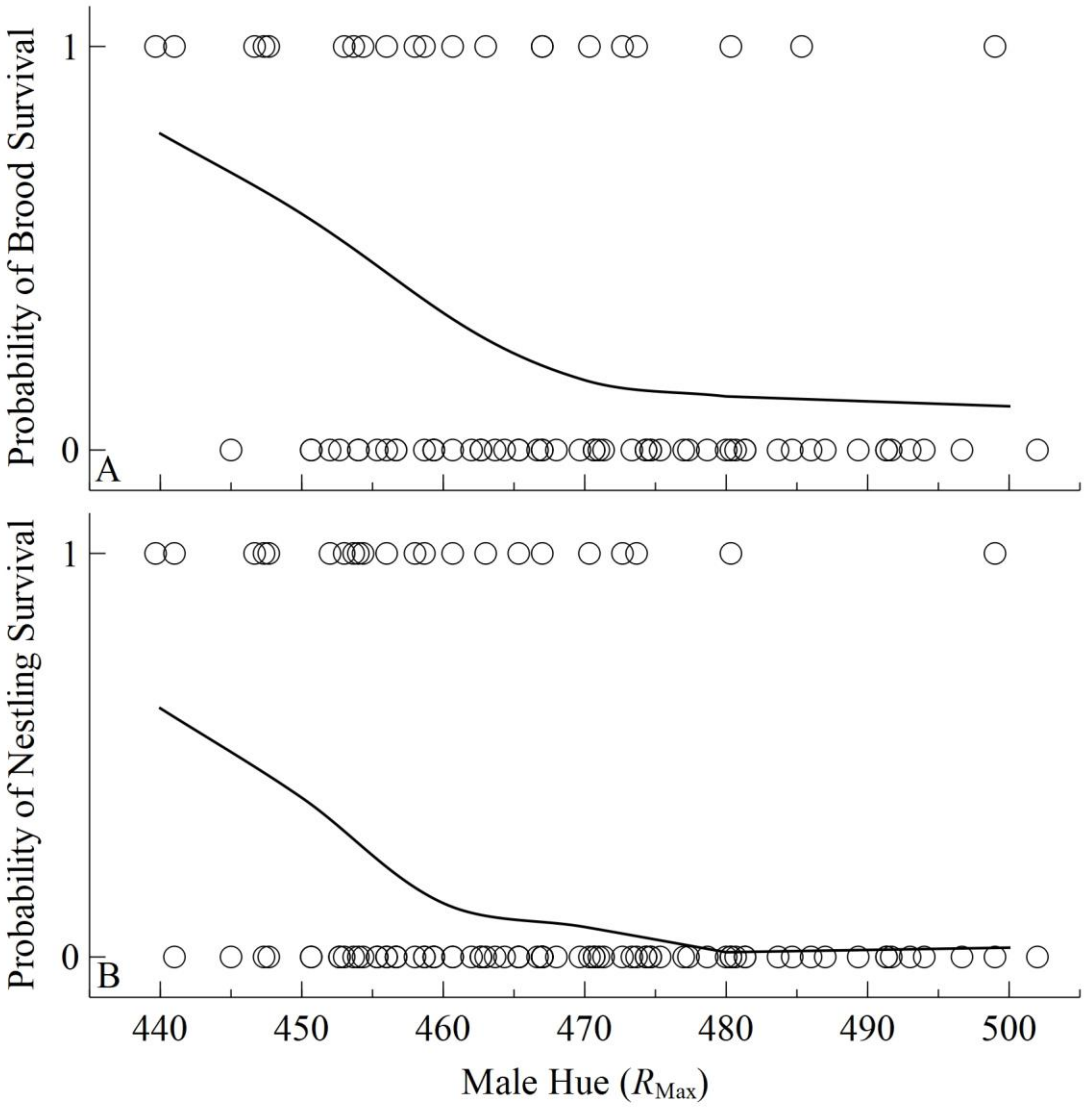

Figure 4.2 The probability that (A) broods and (B) individual nestling tree swallows (*Tachycineta bicolor*) survived a 2-day harsh weather event in June 2016 decreased with male plumage hue, i.e., males with bluer plumage (lower hue values) had higher brood survival than males with greener plumage (higher hue values). See Methods for details of measuring plumage hue. Brood survival was defined as all nestlings dying within a brood (0) or at least one nestling surviving in a brood (1). Nestling survival was defined as 0 when a nestling died and 1 when a nestling survived. The prediction lines were estimated using generalized linear mixed effects models (logit link and binomial family).

Table 4.2 Results of a generalized linear mixed effects model examining the survival of individual nestling tree swallows (*Tachycineta bicolor*) during a 2-day harsh weather event in June 2016 in central British Columbia, Canada.

Response variable	n	Model variables	Estimate	SE	z	P	95% CI	
							Lower	Upper
Nestling survival	411	IVM treatment:						
		0 vs. 1	1.721	1.18	1.46	0.146	-0.60	4.04
		0 vs. 2	3.443	1.21	2.85	0.004	1.08	5.81
		0 vs. 3	3.680	1.23	2.99	0.003	1.27	6.09
		1 vs. 2	1.722	1.17	1.47	0.141	-0.57	4.01
		1 vs. 3	1.958	1.18	1.65	0.098	-0.36	4.28
		2 vs. 3	0.237	0.64	0.37	0.712	-1.02	1.49
		heat treatment:						
		ctl vs. heat	-3.300	1.49	-2.22	0.026	-6.21	-0.39
		age	-1.893	0.49	-3.87	<0.001	-2.85	-0.93
		age^2	0.109	0.03	3.87	<0.001	0.05	0.16
		relative nestling size	0.677	0.27	2.49	0.013	0.14	1.21
		male hue	-0.155	0.04	-3.52	<0.001	-0.24	-0.07
		female brightness	-0.408	0.18	-2.29	0.022	-0.76	-0.06
		female UV chroma	-26.743	10.53	-2.54	0.011	-47.38	-6.11

Note: Nestlings were involved in an experiment that used an anti-parasite drug, ivermectin (IVM), to reduce parasite load within nests. Nestlings from non-experimental broods (0) were not involved in the anti-parasite experiment. Nestlings from control broods (1) received sham-injections of sesame pure oil, while half of the nestlings in IVM broods received control injections of sesame oil (2) and the other half received IVM injections (3); see Methods for details.

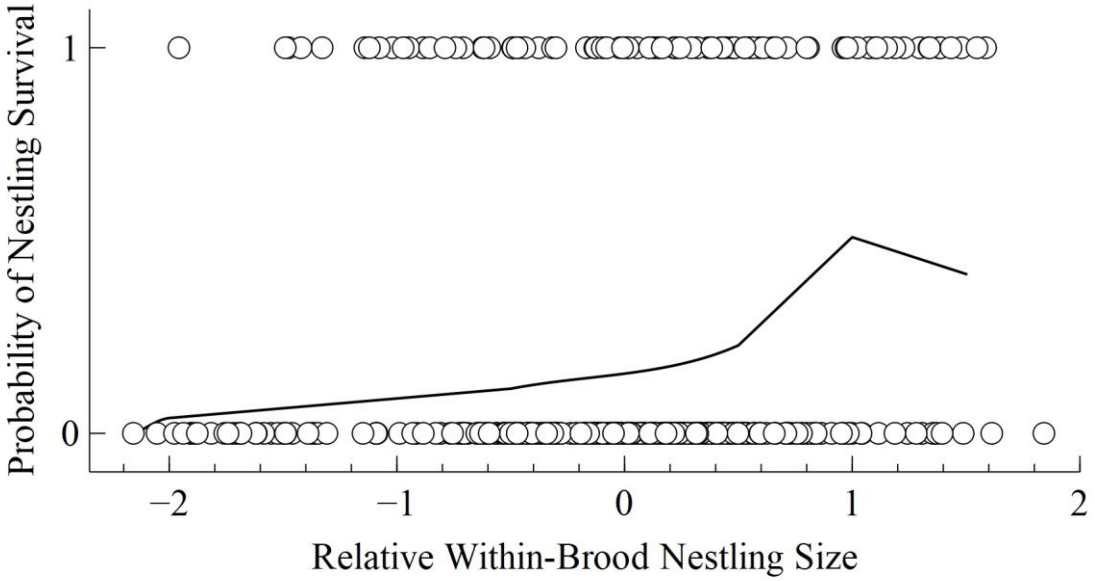

Figure 4.3 The probability of individual nestling tree swallows (*Tachycineta bicolor*) surviving a 2-day harsh weather event in June 2016 was higher for the largest nestlings within a brood and lower for the smallest nestlings within a brood. Survival was defined as 0 when a nestling died and 1 when a nestling survived. The prediction line was estimated using a generalized linear mixed effects model with a logit link and binomial family.

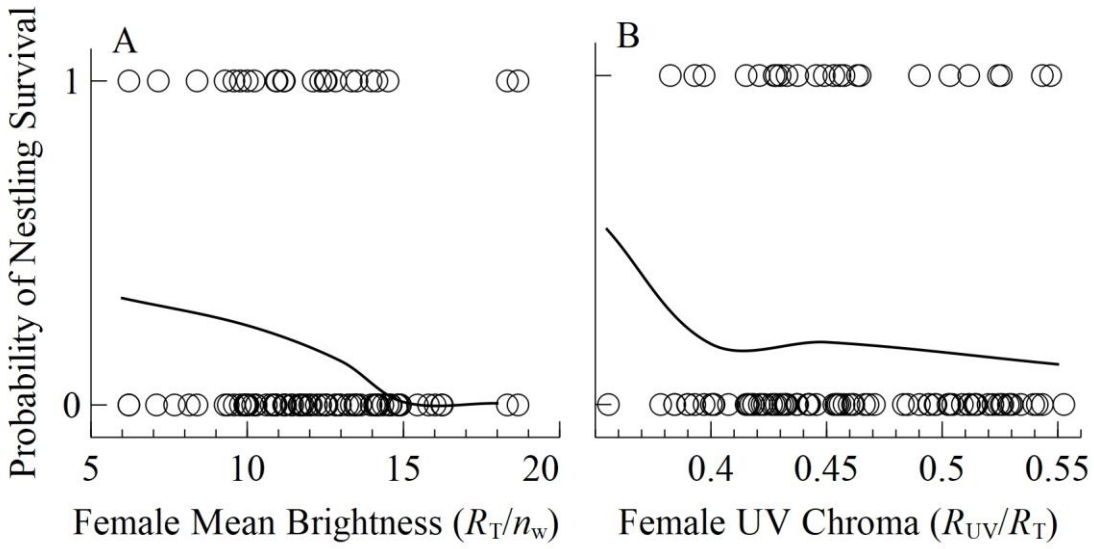

Figure 4.4 The probability of nestling tree swallows (*Tachycineta bicolor*) surviving a 2-day harsh weather event in June 2016 decreased with (A) brightness and (B) UV chroma of parent females. See methods for details of quantifying plumage brightness and UV chroma. Survival was defined as 0 when a nestling died and 1 when a nestling survived. The prediction lines were estimated using generalized linear mixed effects models with a logit link and binomial family.

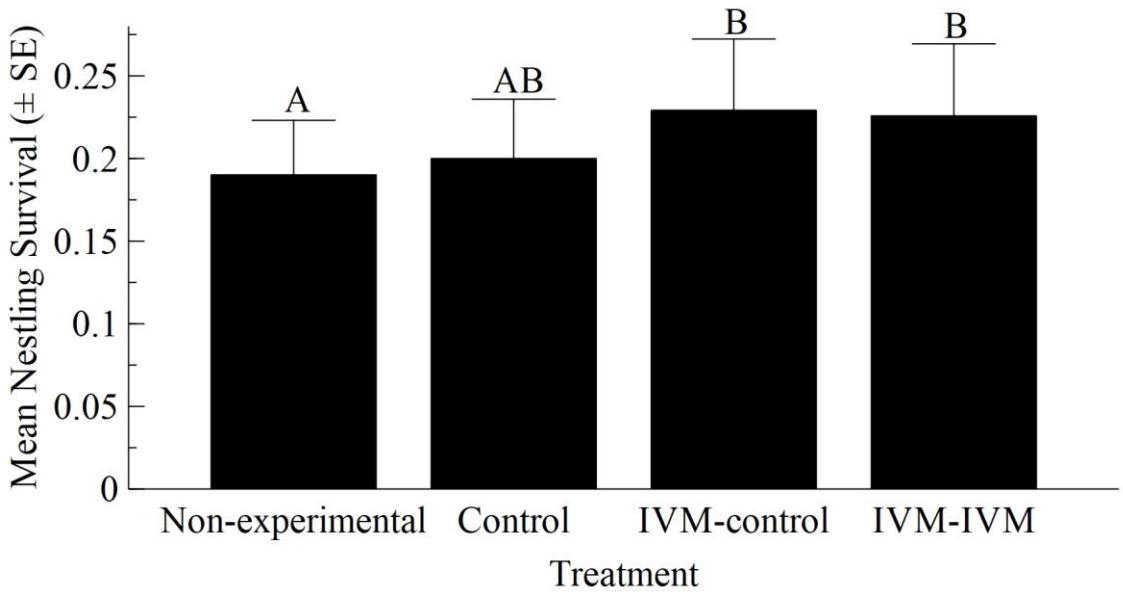

Figure 4.5 Mean survival (± SE) of nestling tree swallows (*Tachycineta bicolor*) during a harsh weather event in June 2016. Nestlings from non-experimental broods were not experimentally manipulated (n = 117 nestlings), while nestlings from control broods received sham-injections of pure sesame oil (n = 120 nestlings). Within ivermectin (IVM) broods, half of the nestlings received injections with a broad-spectrum, anti-parasite drug (IVM-IVM; n = 85 nestlings) and the other half received control injections of sesame oil (IVM-control; n = 89 nestlings); see Methods for details. Treatments that share the same letter were not significantly different (P > 0.05).

significantly from the survival of nestlings from either non-experimental broods or IVM broods. Nestlings from heated nests (reduced ectoparasite loads) had lower survival than nestlings from control nests (natural ectoparasite loads; Fig. 4.6). Morphology of parents, initial female reproductive investment (i.e., total egg mass and mean relative yolk volume), brood size, and relative hatching asynchrony were not significant in predicting nestling survival.

4.5 Discussion

Two factors predicted both brood and nestling survival of tree swallows: brood age and plumage hue of males (Table 4.1, 4.2). Survival displayed a curvilinear relationship with brood age and negative relationship with plumage hue of males. Five additional factors predicted nestling, but not brood, survival: IVM treatment, heat treatment, relative nestling size, and brightness and UV chroma of female plumage (Table 4.2). Nestlings from broods where half the nestlings received IVM injections were more likely to survive than nestling not involved in the experiment. In contrast, nestlings from parasite-free nests (i.e., heated nests) were less likely to survive than nestlings from parasite-infested nests (i.e., control nests). Nestling survival was positively related to relative nestling size and brightness and UV chroma of female plumage. The relationship between survival and brood age was curvilinear, such that middle-aged broods and nestlings were less likely to survive than younger or older broods and nestlings (Fig. 4.1). In tree swallows, the age of physiological endothermy is 9.5 days, but effective homeothermy can range from 4–8 days once brood size is accounted for (Dunn 1979). Therefore, middle-aged nestlings are beginning to thermoregulate, an energetically expensive activity, but have poorly developed feathers and a limited capacity to retain the heat they generate. This makes middle-aged nestlings particularly vulnerable to cold periods. For nestling tree swallows, Boyle et al.

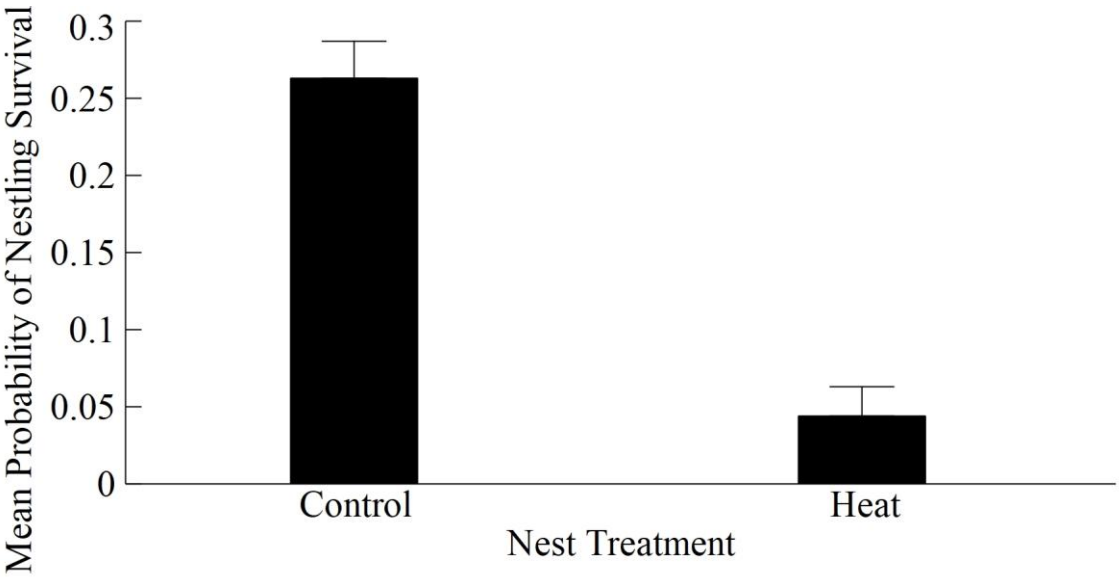

Figure 4.6 Mean nestling survival (± SE) of tree swallows (*Tachycineta bicolor*) during a harsh weather event in June 2016. Nestlings from nests that were heated in a microwave oven repeatedly to kill all nest-dwelling ectoparasites (heat nest treatment; n = 17 broods, 102 nestlings) had significantly lower survival than nestlings from unheated nests (control nest treatment; n = 57 broods, 309 nestlings).

(2013) identified the most at-risk age of mortality during cold snaps as 6–9 day old. This agrees with my results, although I also observed low survival in nestlings up to 12 days old (Fig. 4.1).

Brood and nestling survival decreased with male plumage hue (Fig. 4.2). This supports the 'good parent hypothesis', which predicts that females use ornaments displayed by males to select higher quality mates that provide better parental care (Hoelzer 1989). Males with bluer plumage (lower hue values) may have provided better care for the nestlings, increasing the brood's chance of surviving. As male tree swallows do not brood the nestlings, this better care would have to be provided as improved provisioning or acquisition of higher quality territory. Plumage ornamentation of male birds has been positively associated with feeding rate (e.g., Pagani-Núñez and Senar 2014), the number of young fledged (Silva et al. 2008; Moreno et al. 2013), brood mass (Saetre et al. 1995), and mass of young at fledging (Siefferman and Hill 2003). Specifically in my population, older male tree swallows were bluer and brighter than younger males (Bitton and Dawson 2008). This result appeared to be caused by individuals increasing in brightness over time and the higher likelihood of bluer males returning to the breeding site. Thus, bluer males may be of higher quality and have more breeding experience, which could have improved the chance of their young surviving compared to younger, less experienced males with greener plumage. Alternatively, younger males with greener plumage may have favoured a self-maintenance strategy to improve their chance of future reproductive success, whereas older males with bluer plumage may have invested more in their current reproductive attempt. Furthermore, adults may adjust their level of parental care in response to the quality of plumage ornamentation displayed by their mate. In a meta-analysis, Horváthová et al. (2012) concluded that when paired with a more attractive mate, females in general invested more into reproduction. Therefore, the females paired with males displaying more blue plumage

also may have provided better care to the nestlings, thereby increasing the brood/nestling's chance of surviving. In tree swallows, however, Dakin et al. (2016) showed that adults (females and males) paired with a partner with greener plumage, had higher feeding rates, which contradicts the previous possibility. It is important to note, however, that Dakin et al. (2016) quantified provisioning when offspring were only 3 days old and demands on parents would have been small. Moreover, they quantified rate of feeding, but not the quantity or quality of food that was delivered. Thus, bluer males may feed at a lower rate, but provide young with more food and/or higher quality food (Twining et al. 2016).

Female plumage brightness and UV chroma both negatively predicted nestling survival (Fig. 4.4), contradicting the 'good parent hypothesis' that predicts that male birds select a mate using female plumage ornamentation as a signal of the quality of parental care the female can provide (Owens and Thompson 1994; Johnstone et al. 1996). Female tree swallows with brighter, bluer plumage, however, have been shown to produce nestlings with lower body condition (Bentz and Siefferman 2013), supporting my results. In contrast, in my population of tree swallows, females with bluer plumage produced more fledglings than females with greener plumage (Bitton et al. 2008), which appears to contradict my findings. It is important to recognize, however, that Bitton et al. (2008) examined general reproductive success, whereas I examined reproductive success during a specific, unfavourable situation. Alternatively, if males reduced their care in response to female brightness and/or UV chroma (and females were unable to compensate), then nestling survival may have been negatively affected. Previous studies of other species have documented males decreasing (Limbourg et al. 2013) or increasing (Mahr et al. 2012) their level of parental care when paired with females displaying higher quality ornamentation. For example, when the UV reflectance of crown feathers in female blue tits

(*Cyanistes caeruleus*) was experimentally reduced, male feeding rate was lower than males paired with sham-treated, control females (Mahr et al. 2012). A previous experiment in my population of tree swallows found that manipulating female plumage brightness, either making them brighter or duller, did not influence male feeding rate (Berzins and Dawson 2016). Unfortunately, since I did not directly measure parental effort, I cannot identify how adults with certain plumage characteristics produced nestlings with higher survival during a harsh weather event.

A nestling's chance of survival increased with its relative size within the brood, with the smallest nestling having the lowest chance of survival and the largest nestling having the highest chance of survival (Fig. 4.3). With the fewest body reserves to generate heat and endure starvation, and the least developed feathers to retain body heat, the smallest nestling within a brood is often expected to be the first to die when conditions are poor (the 'brood reduction hypothesis'; Lack 1947; Howe 1978).

Treatment of half of the nestlings in a brood with a broad-spectrum, anti-parasite drug, ivermectin, resulted in higher survival of nestlings than those from non-experimental broods (Fig. 4.5). Following two field seasons of the IVM experiment, analyses found that nestlings from ivermectin-treated broods faced marginally lower parasite loads (number of larval blow flies per nestling) and had longer and faster growing flight feathers, higher haemoglobin concentrations, and greater fledging success than nestlings from control broods (Chapter 2). Fewer parasites in IVM broods may have meant nestlings had more resources available to endure starvation and, for older nestlings, to invest in thermoregulation. Haemoglobin concentration in birds is related to their oxygen-carrying capacity (Minias 2015) and thus, for nestlings that had begun thermoregulating, the higher haemoglobin concentrations of nestlings from IVM broods

may have improved their thermoregulatory capabilities compared to nestlings from control and non-experimental broods (Petit and Vézina 2014). Therefore, I would have expected the survival of nestlings from IVM broods to not only be greater than non-experimental nestlings, but also greater than nestlings from control broods, but this was not the case. Possibly the carrier oil used to deliver the IVM, which all nestlings involved in the experiment received injections of, provided the nestlings with additional nutrients and energy, making them more resilient to the harsh weather. This does not seem likely, however, because nestlings received less than 30 μL of oil. Alternatively, there may have been a lack of statistical power and differences may have been detected if sample sizes were larger.

Nestling survival was lower in heated nests than unheated nests (Fig. 4.6). This contradicts my prediction that lower parasite loads would permit nestlings to allocate more energy to coping with the effects of harsh weather. Rather, my results suggest that nestlings exposed to higher parasite loads were more resilient to harsh weather. This result, however, may be an artifact of site differences. The heat treatment was only applied at one site, Western, which experienced higher rates of mortality than Stewards (Western: 86% brood mortality, Stewards: 49% brood mortality). Breeding chronology was approximately one week ahead at Western compared to Stewards (mean brood age at time of harsh weather event ± SE; Stewards = 5.7 ± 0.45, Western = 10.6 ± 0.53). Thus, the relationship between heat treatment and survival may have been a consequence of more middle-aged nestlings occurring in heated nests due to study design and temporal differences between the two field sites. The results of the IVM treatment, which was applied evenly across both sites and suggest that nestlings exposed to lower parasite loads may have higher survival, support this possible explanation.

In conclusion, I have identified several possible factors influencing survival of broods and individual nestlings in a species of aerial insectivorous bird during a single harsh weather event. Two of the key factors (age and plumage hue of the male) predicted survival at both the brood and nestling level, while the other factors only predicted nestling survival (relative size of the nestling, female brightness and UV chroma, and two different anti-parasite treatments). Aerial insectivores are declining across North America (Nebel et al. 2010) and my analysis of survival during harsh weather may be important within the broader context of climate change. As climate change progresses, it is predicted that the frequency of extreme weather events will increase (IPCC 2014). Thus, the knowledge gained from this study, and research like it, may become crucial for understanding dynamics of aerial insectivorous bird populations in the future.

5. General Discussion

During the nestling period of altricial birds, when young undergo an intense phase of rapid growth and development, nestlings are often exposed to a diversity of nest-dwelling, blood-feeding ectoparasites. During this energetically expensive stage, parasites can negatively affect nestlings (e.g., Merino et al. 2001; Fessl et al. 2006). Although the effects of parasites on nestlings have been studied for at least 40 years (e.g., Whitworth 1976), most experimental manipulations of parasite load have been performed at the nest-level (reviewed in Hund et al. 2015), with far fewer studies manipulating parasite load at the individual-level (e.g., Reed et al. 2012; Burthe et al. 2013). Individuals, however, can differ in their susceptibility to parasites due to both intrinsic (e.g., sex; O'Brien and Dawson 2013) and extrinsic (e.g., environmental conditions; Allander 1998) factors and therefore, I would expect not only broods to differ in their response to parasites, but also nest mates. Variation in susceptibility to parasites within broods may be particularly pronounced in bird species that hatch asynchronously, which results in the establishment of size hierarchies among siblings (e.g., Bryant 1978; Stokland and Amundsen 1988; Magrath 1992). These size hierarchies increase the morphological (e.g., Zach 1982) and physiological (e.g., Martínez-Padilla et al. 2004), including immunological (e.g., Saino et al. 2001), variation within broods, which could exaggerate the differences in parasite susceptibility observed among nest mates. The objective of this study was to better understand nestling susceptibility to parasites at the brood- and nestling-level in tree swallows, an asynchronously hatching bird. This was achieved by 1) manipulating susceptibility to parasites by treating individual nestlings with a broad spectrum, anti-parasite drug, ivermectin (IVM), and 2) manipulating haemoglobin concentration, a common effect of haematophagous ectoparasites,

using the chemical phenylhydrazine hydrochloride (PHZ). For both experiments, the effects of the treatment on morphology, physiology, and survival, both among and within broods, were measured and analyzed. In addition, nestling susceptibility to parasites within the context of a harsh weather event was opportunistically examined.

Haemoglobin concentration of day 12 nestlings was higher when the ectoparasite load was reduced, either by injecting partial broods with IVM (Chapter 2), or by heating nests in a microwave oven (Chapter 3). This agrees with many studies across a wide variety of bird species that have found a negative relationship between the haematophagous ectoparasite load and nestling haemoglobin concentration (reviewed in Minias 2015). This suggests that ectoparasites remove a sufficient volume of blood from young birds, such that nestlings are unable to generate enough haemoglobin to maintain normal concentrations. Nestlings injected with PHZ on day 6 showed no change in haemoglobin concentration to day 8, whereas control-injected nestlings showed the typical increase in haemoglobin concentration with age from day 6 to 8 (Chapter 3). The combination of the PHZ treatment with a nest heating treatment suggested that ectoparasites had less of an effect on haemoglobin concentration at younger ages (day 6 to 8) compared to at older ages (day 12). This demonstrates that the effects of parasites on nestlings can be age-dependent. For example, nestlings may pay higher costs of parasitism during periods of high growth and development for the nestling and/or the parasite. Larval blow flies, which progress through three instars, grow in size over the nestling period and thus, require larger blood meals later during their development (Whitworth and Bennett 1992).

Development of the flight feathers was positively affected by both the IVM treatment (marginal reduction in parasite load; Chapter 2) and the combination of the PHZ and nest heating treatments (significant reduction in parasite load; Chapter 3). Thus, nestlings exposed to fewer

ectoparasites appeared to have more energy and resources available to invest in feather growth. These results support that feather growth is a more plastic trait that can be influenced by rearing conditions, whereas structural traits, like head-bill, may be more genetically fixed (Wiggins 1990) or constrained by calcium availability (Dawson and Bidwell 2005). Interestingly, nestlings with experimentally reduced haemoglobin concentration (i.e., PHZ-injected nestlings) also tended to have longer flight feathers near to fledging, regardless of parasite load (Chapter 3). Potentially, this suggests that a sudden reduction in haemoglobin concentration early in the nestling period may act as a physiological signal of high parasite loads that could result in faster feather growth, allowing earlier fledging and escape from a parasite-rich nest environment. Faster feather growth in response to ectoparasites has been previously documented in barn swallows (*Hirundo rustica*; Saino et al. 1998) and the reduction in haemoglobin concentration as a potential mechanism definitely warrants further investigation. As I have shown that PHZ can be effectively used in wild nestling birds (to my knowledge, the first study to do this), PHZ provides an excellent tool to test this potential mechanism, as well as other effects of reduced haemoglobin, experimentally.

Two results, the effect of the IVM treatment on mass near to fledging (Chapter 2) and the combined effects of the PHZ and nest heating treatments on the growth rate of the ninth primary feather (Chapter 3), depended on relative brood asynchrony. Mass increased with relative brood asynchrony, but only when parasite loads were higher (i.e., control broods) not lower (i.e., IVM broods; Chapter 2). This suggests that brood asynchrony provided some benefit under high parasite conditions and this benefit was no longer present when parasite load was reduced. This positive relationship between mass near to fledging and degree of asynchrony has been observed across a number of bird species (reviewed in Amundsen and Slagsvold 1991). In contrast, the

growth rate of ninth primary was negatively related to relative brood asynchrony when nestlings in heated nests (i.e., parasite-free nests) received control injections (i.e., haemoglobin concentration was not reduced), whereas nestlings in all other treatment groups of the haemoglobin reduction experiment showed no relationship between brood asynchrony and growth rate of the primaries (Chapter 3). Unfortunately, it is more difficult to assess this pattern, as faster flight feather growth could be advantageous (e.g., indicating that nestlings have more resources available to allocate to growth and development) or a constraint (e.g., if nestlings are growing flight feathers more quickly, potentially at the cost of some other aspect of development, to fledge earlier and leave a poor nest environment, such as intense sibling competition). Although the interpretation of this latter interaction is difficult, both these results demonstrate that the amount of variation within a brood can alter the effects observed and, particularly the former of these two results, highlights the importance of considering the ectoparasite environment when studying the effects of hatching asynchrony.

The proportion of young fledged from a brood tended to be higher both in nests where the parasite load was marginally reduced (i.e., IVM broods; Chapter 2) and nests with no parasites (i.e., heated nests in the haemoglobin reduction experiment; Chapter 3). This suggests that parasites in my population removed enough energy and resources from the nestlings to reduce their chance of fledging. Reduced fledging success due to nest-dwelling ectoparasites has been documented across a number of bird species (e.g., Richner et al. 1993; de Lope et al. 1993; Merino et al. 2001; Fessl et al. 2006), but not consistently in tree swallows (e.g., Shutler et al. 2004; DeSimone et al. 2018) or other species (e.g., Johnson and Albrecht 1993; Hannam 2006). This may mean that at my study sites parasite populations were large enough to have an impact (McKilligan 1996), environmental conditions were conducive to allow greater virulence of

parasites (Allander 1998), or adults did not compensate for the energy their offspring lost to parasites (Christe et al. 1996a). In agreement with this last suggestion, I measured the feeding rate of adults when nestlings were 8 days old and found no difference between parasite-free (i.e., heated nests) and parasite-infested nests (i.e., control nests; Chapter 3). In the haemoglobin reduction experiment, the fledging success of parasitized broods increased as the season progressed, but without a concurrent decrease in parasite load (Chapter 3), suggesting that some environmental component (e.g., the quality or quantity of aerial insects available; Twining et al. 2016) may have increased over time at the Western field site during the 2017 field season. This seasonal variation, however, was not observed in the IVM experiment (Chapter 2), which was performed at the Stewards field site over two years (2016 and 2017). Thus, this interaction may have been an artifact of the small sample size of the PHZ experiment.

Under average weather conditions, larger-sized nestlings within a brood were more likely to fledge than their smaller nest mates, but only when parasite loads were lower (i.e., IVM broods), not higher (i.e., control broods; Chapter 2). This suggests that larger nestlings within broods benefited more than smaller nestlings from the anti-parasite treatment and thus, may be more susceptible to parasites. Further work needs to be done to determine whether larger nestlings are more susceptible because parasites are preferentially feeding on them (e.g., Roulin et al. 2003; Valera et al. 2004; Walker and Rotherham 2011) or because larger nestlings are less tolerant of parasitism compared to smaller nestlings within a brood. Within the context of a two-day harsh weather event, regardless of their position in the brood size hierarchy, nestlings were more likely to survive if they, or their nest mates, received IVM injections compared to nestlings not involved in the experiment. This suggests that parasites can affect nestling survival during

inclement weather, as has been previously shown (Dufva and Allander 1996; Allander 1998; Merino and Potti 1996).

In tree swallows, hatching asynchrony creates size hierarchies that increase the variation within broods. Although I was predominantly interested in investigating what effect that variation may have on nestling susceptibility to parasites, I also wanted to document what other traits during the nestling stage may be affected by the initial establishment of a size hierarchy. Relative nestling size at 2 days post-hatch positively predicted a number of morphological traits, including nestling mass and length of head-bill at day 6, length of ninth primary feather at day 8, length of ninth and head-bill at day 16, and growth rates of mass, ninth primary feather and head-bill (Chapter 2 and 3). Haemoglobin concentration at day 12 was also positively related to relative nestling size at day 2 (Chapter 2 and 3). Lastly, relative nestling size positively predicted the fate of nestlings, during both good (Chapter 2) and poor (Chapter 4) weather conditions. This summary suggests that, within broods, nestlings that are larger shortly after hatching grow faster, have a greater oxygen carrying capacity, and obtain longer flight feathers and structural size near to fledging compared to their smaller nest mates. Perhaps as a result of these morphological and physiological advantages, larger nestlings within a brood are also more likely to fledge successfully relative to smaller nestlings.

In conclusion, I have found that differences in nestling susceptibility to parasites, both within and among broods, can affect morphology (particularly the development of flight feathers), physiology (haemoglobin concentration) and survival (both during average and harsh weather conditions) of nestling tree swallows. I also experimentally manipulated a common negative effect of haematophagous parasites (reduced haemoglobin concentration) to test if it causes other negative effects associated with parasitism, but did not find much support for this,

possibly because nestling haemoglobin was not experimentally reduced for a long enough period of time due to fast recovery of haemoglobin by nestlings. Future research should focus on 1) identifying whether or not larval blow flies preferentially feed on certain nestlings within tree swallow broods, and 2) quantifying how immune response varies within and among broods, preferably using a blow fly specific assay (e.g., DeSimone et al. 2018). Building off of my research, these key steps would greatly expand our understanding of nestling susceptibility to parasites and hopefully, reveal whether greater susceptibility to parasites is due to host selection by ectoparasites or differences in nestling immune response.

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